Matrix-Assisted Laser Desorption and Ionization as a Mass Spectrometric Tool for the Analysis of Poly[(*R*)-3-hydroxybutanoates]. Comparison with Gel Permeation Chromatography

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ABSTRACT: Mass spectrometric analysis of oligomers derived from poly[(*R*)-3-hydroxybutanoate] (PHB), containing up to 96 monomer units, showed that molecules of this polymer with $m < 10\,000$ Da can be easily detected using the technique of matrix-assisted laser desorption and ionization mass spectrometry (MALDI-MS). This method also allowed the characterization of oligomer distributions obtained by partial depolymerization of PHB through pyrolysis or treatment with bases. Comparison of the results with the data from gel chromatography showed that, with some limitations, both methods yield similar results.

Introduction

Polyhydroxyalkanoates are a class of biopolymers that accumulate in several microorganisms as storage compounds, if certain nutrients are limited during growth and carbon sources are available in excess.

Because of their production from naturally growing resources and their biodegradability and biocompatibility, polyhydroxyalkanoates are subject to many expectations. These are well reflected by numerous reviews[1–3] and the steadily increasing number of patents.[4] Variation of the microorganism or the carbon sources makes copolymers with different compositions accessible. These are of potential interest for the production of tailor-made biodegradable plastics.

Up to now only the homopolymer PHB and the copolymer PHB/HV containing (*R*)-3-hydroxybutanoate and a maximum of 20% (*R*)-3-hydroxyvalerate (sold as "BIOPOL") are commercially available.[5] The copolymer is presently used for packaging purposes. Its uses in manufacture of fibers and films are potential applications.[6]

The molecular weight of PHB produced by *Alcaligenes eutrophus* is approximately 7.5×10^5 g/mol. Especially because of its stereoregular (*all-R*) configuration the homopolymer PHB is of great importance as starting material for monomeric chiral building blocks.[3,7]

Oligomers of PHB, which may have applications (a) as plasticizers for the high molecular weight material, (b) for drug delivery systems,[2,8] or (c) for the synthesis of block copolymers,[9] can be obtained from PHB by three different methods: pyrolysis,[10] degradation in the presence of lithium amide bases,[11,12] or partial transesterification.[13] Such rather broadly distributed oligomers may be characterized by light scattering,[14–16] sedimentation equilibrium,[17] determination of colligative properties (membrane or vapor pressure osmometry, ebullioscopy, or cryoscopy), end group analysis,[11] HPLC,[18] viscosimetry,[17] or gel permeation chromatography (GPC) (for PHB see ref 11). The last three methods require—as relative methods—calibration with standards of known molecular weight (absolute values from GPC may be obtained using the universal calibration procedure[19] or a light scattering detector;[16] with the latter it is even possible to derive data concerning the solution structure). All methods, with the exception of HPLC and GPC, yield averages of the molecular weight ($\bar{M}_w$, $\bar{M}_n$, $\bar{M}_v$, ...) and no information about the distribution.

Use of mass spectrometric analysis for the determination of molecular weight distributions (MWD) is becoming more and more established,[20] especially due to the development of the so-called soft ionization techniques.[21] In contrast to the methods mentioned above, the experimental procedures are simple and information concerning chemical structure, purity, and distribution of monomeric units in a copolymer is obtained besides the MWD.

On the other hand, it is important that interfering fragmentation processes are excluded and that the sensitivity of the mass spectrometer does not decrease with increasing molecular weight.

Up to now there are only two publications concerning mass spectrometric analysis of oligomer distributions of polyhydroxyalkanoates. Seebach *et al.* analyzed trimodal distributions of oligomers, obtained by treating PHB or PHB/HV with lithium amide bases in tetrahydrofuran at low temperatures, using plasma desorption and ionization mass spectrometry (PD-MS) (first maximum 1600 Da, second 2700 Da, third 4200 Da).[11] Oligomers with higher molecular masses (>2000 Da) were detected with lower sensitivity, as shown by comparison with GPC. Thus, the number average molecular weight ($\bar{M}_n$) calculated from PD-MS was approximately 1000 g/mol smaller than the value determined by vapor pressure osmometry, end group analysis, or GPC. Nevertheless only peak heights and not peak areas were used for the evaluations; so the deviations may result from this calculation method as well. The authors have also shown that commercial PHB/HV is a statistic copolymer.

Montaudo *et al.* analyzed similar distributions of oligomers obtained by partial methanolysis or pyrolysis of PHB or PHB/HV, using fast atom bombardment mass spectrometry (FAB-MS). They were able to distinguish between a pure copolymer and a blend of different random copolymers. They also showed that the monomeric units are randomly distributed in the bacterial copolymer PHB/

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HV. However, the detection limit of 2000 Da was rather low.[22]

Matrix-assisted laser desorption and ionization (MALDI) was discovered by Karas *et al.*[23] in 1988, and the basic technique was extended by several workers.[24] Their discoveries suggested applicability of this method in various fields of chemistry, and hitherto unavailable mass spectrometric data were obtained. The absence of substantial fragmentation, the extremely high sensitivity (pmol to fmol range), easy sample handling, and short analysis time make MALDI a nearly ideal technique for the analysis of complex polymer mixtures. Already in 1988 Tanaka was able to analyze synthetic polymers with masses up to 20 kDa and proteins even in the 100-kDa region.[25] Recently it was shown that MALDI-MS can provide mass spectra over a large mass range from 1 kDa up to 70 kDa for several different polymers.[26]

This work will show a qualitative analysis of oligomers derived from PHB, and an attempt for quantitative analysis of oligomer distributions will be made by comparing the mass spectrometric result with results from GPC. There are already several publications about correlation of GPC and MS, however, with different polymers and techniques somewhat different from MALDI-MS.[27]

Experimental Section

Synthesis of PHB Oligomers. The monodisperse PHB derivatives **A** were synthesized by following a segment condensation strategy. Starting with the monomers (*R*)-3-(benzyloxy)-butyric acid and *tert*-butyl-(*R*)-3-hydroxybutyrate, a benzyl ether/*tert*-butyl ester protected dimer was obtained by reaction of the acid chloride (first treatment of the benzyloxy acid with $(COCl)_2$, in CH_2Cl_2, and then addition of the *tert*-butyl ester and pyridine). The benzyl protective group was removed from half of the resulting oily product by hydrogenolysis (H_2, Pd–C, ethanol or DMF). The other half was treated with CF_3COOH/CH_2Cl_2 for removal of the carboxylic acid protecting group. Coupling of these selectively protected dimers was accomplished by an acid chloride coupling as described above for the synthesis of the dimer. Following this scheme, the compounds **A** with 16, 32, 64, and 96 monomeric units were synthesized[28] (the latter by coupling of the benzyl ether-protected 64mer with the *tert*-butyl ester-protected 32mer).

Weighing. A mixture of 2 mg of each of the 16-, 32-, 64-, and 96mer dissolved in 5 mL of CH_2Cl_2 (first determination; on a laboratory analysis scale Mettler H311) or a mixture of 1.0028 mg of 16mer, 3.3168 mg of 32mer, 0.8353 mg of 64mer, and 0.3992 mg of 96mer (second determination; on a microanalytical scale Mettler ME22/BA25) was used for the MALDI-MS and GPC analysis.

Partial Depolymerization of PHB with LiHMDS/LiCl. A suspension of 3.0 g of reprecipitated PHB[11] (34.8 mmol HB-units) and 3 equiv of LiCl (4.43 g, 105 mmol) in 300 mL of THF (destilled over sodium/benzophenone) was stirred for 2 h at room temperature, and then cooled to −74 °C. Lithium hexamethyldisilazide (LiHMDS, 3 equiv) generated from 22.5 mL (108 mmol) of hexamethyldisilazane in 10 mL of THF and 68 mL (109 mmol) of *n*-butyllithium (1.6 M in hexane) was added as a cold suspension (−74 °C) to the PHB suspension during 10 min through Teflon tubing; the mixture was stirred at −70 °C for an additional 10 min. A saturated aqueous NH_4Cl solution (30 mL) was added to the colorless, turbid reaction mixture, which was then allowed to warm to room temperature. After evaporation of the organic solvents, the aqueous residue was extracted three times with $CHCl_3$. The combined organic layers were washed successively with saturated aqueous $NaHCO_3$ (twice), 2 N HCl, and saturated aqueous NaCl and then dried with $MgSO_4$. After evaporation, diethyl ether was added to the solid residue and the suspension was stirred vigorously; the solid was filtered off and washed with diethyl ether. Drying under *high* vacuum gave 2.24 g (79% yield) of a white powder.

Treatment of 1.5 g of this product under the same conditions yielded 1.19 g (79% yield) of a white powder; 775 mg of this twice partially depolymerized PHB was again treated as above and afforded 515 mg (66% yield) of a white powder **B1** with a number average degree of polymerization ($\bar{X}_n$) of 20 (by end group analysis).

Separation on a Preparative Scale. A 150-mg amount of the oligomer mixture **B1** was separated into 74 mg of a high molecular weight and 63 mg of a low molecular weight fraction by chromatography on Sephadex LH60 using CH_2Cl_2 as eluent (column diameter 50 mm, bed height 600 mm). A fraction (11 mg) containing both high and low molecular weight oligomers in approximately the same amounts (according to GPC analysis) was also isolated.

Depolymerization of PHB by Pyrolysis. PHB (10 g) was heated to 210 °C for 3 h in an argon atmosphere, cooled to room temperature, and dissolved in $CHCl_3$. After treatment with activated charcoal, the solvent was removed and the residue was suspended in diethyl ether and stirred vigorously. The solid was filtered off and washed with diethyl ether. Drying under high vacuum yielded 7.13 g (71% yield) of a white powder **B2** with $\bar{X}_n$ = 20 (by end group analysis).

Heating the same amount of PHB under similar conditions for 2 h yielded 9.00 g (90% yield) of a white powder **B3** ($\bar{X}_n$ = 60; by end group analysis).

End Group Analysis. The sample was dissolved in $CHCl_3$ and treated with an excess of diazomethane in diethyl ether. The solvent was removed after fading of the yellowish color.

The number average molecular weight ($\bar{M}_n$) was determined from the ratio of the area under the signals for the methyl ester and the 3-methyl groups ($C-CH_3/O-CH_3$) obtained by ^1H-NMR (Varian Gemini *200*, 200 MHz, solvent $CDCl_3$).

Mass Spectrometer. The mass spectrometer employed was a linear time-of-flight MS (TOF-MS) with a 1.7-m flight tube. For matrix-assisted laser desorption and ionization (MALDI), the sample is embedded in a solid matrix of *e.g.* 2,5-dihydroxybenzoic acid, with the matrix being present in an 100- to 10 000-fold excess. A short pulse of laser light (3 ns, 3–10 μJ, 337 nm) hits the probe. The light is absorbed by the matrix, which in turn fragments heavily, its crystal structure is destroyed, and the embedded sample molecules are released into the gaseous phase. During this process, whose details are not well understood, charged species develop by loss or addition of a proton (M + H or M − H). The ions are then accelerated in a TOF-MS. The masses are calculated by the time difference between the laser pulse and the time the ions arrive at the detector.

All measurements were done on a prototype (LDI-1700) instrument from Linear Scientific, Inc., Reno, NV. The mass spectrometric resolution ($m/\Delta m$) for the PHB's was 300–500 (fwhm). The detection limit was about 200 fmol.

The samples were prepared for the measurements in the following way: A 0.1 mM solution (10 μL) of the sample in $CHCl_3$ was mixed with a solution of 100 mM 2,5-dihydroxybenzoic acid (with sinapinic acid as matrix no ion signals from the oligomers were detected) in 50% acetonitrile, 45% ethanol, and 5% water (10 μL). A sample (1 μL) of the mixture was applied on the probe tip, the solvent was evaporated under vacuum (1 min), and then the probe was inserted into the mass spectrometer.

Gel Permeation Chromatography. Gel Permeation Chromatography was performed with the Waters HPLC-GPC system (Waters 600E Multi Solvent Delivery System). Detection was done with a Waters 410 Differential Refractometer. Chromatography was done using a series of three GPC columns (Shodex K-802, K-802.5, K-803; stationary phase of styrene–divinylbenzene copolymer) with $CHCl_3$ (stabilized with amylene) as eluent at 35 °C. Calibration was performed using the synthetic PHB derivatives of type **A** with defined molecular weights. Calculations ($\bar{M}_w$, $\bar{M}_n$, $\bar{X}_n$, $\bar{M}_w/\bar{M}_n$) were done by numerical integration using a Waters 746 Data Module with GPC Package.

Results

During our studies of the structure and synthesis of a supposed nonproteinogenic ion channel built of a wrapping PHB helix with 120–200 monomeric units (10 000–17 500 g/mol) surrounding an inner chain of polyphosphate, as

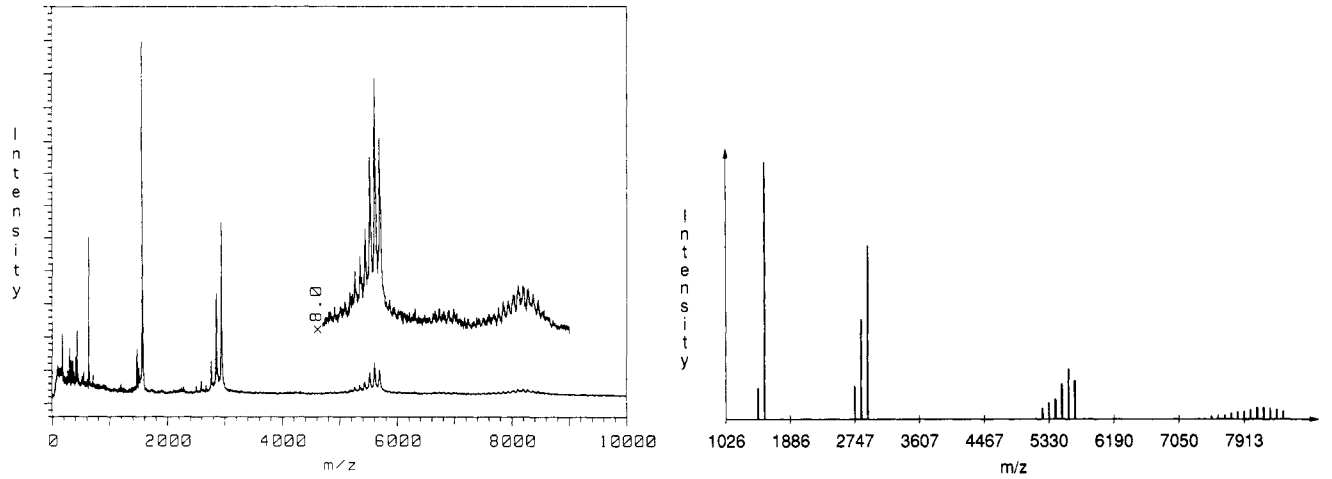


Figure 1. Positive ion MALDI-MS of a mixture of equal masses (see Experimental Section, first determination) of the 16mer, 32mer, 64mer, and 96mer of the PHB derivatives of type A recorded with 2,5-dihydroxybenzoic acid as matrix. The ions below m = 800 Da are from the matrix. On the right side is the line spectrum calculated from the MS using the corrections mentioned in the text.

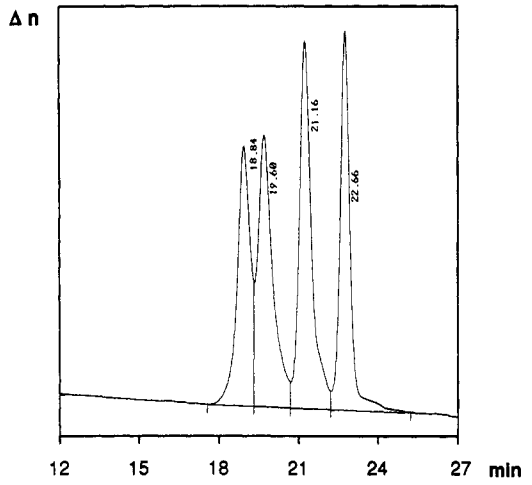
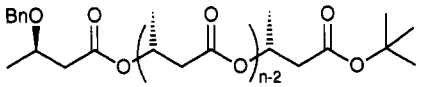


Figure 2. GPC of a mixture of equal masses (2 mg of each compound; see Experimental section, first determination) of the 16mer, 32mer, 64mer, and 96mer of the PHB derivatives type A. (Δn: Refractive index difference between sample and reference.)

proposed by Reusch,[29] we synthesized a number of PHB oligomers (type A) with defined chain lengths consisting



A

of up to 96 units. With a defined mixture of these oligomers changes of sensitivity with increasing molecular weights in the MALDI-MS could be studied. With the oligomers of defined chain length it was also possible to obtain a reliable calibration of GPC measurements. Both points are essential for comparison of GPC and MALDI-MS methods for the analysis of oligomer distributions.

The MALDI-MS of a mixture of equal masses of oligomers with chain lengths of 16, 32, 64, and 96 units is shown in Figure 1 (m in Da; 16mer, 1540; 32mer, 2918; 64mer, 5677; 96mer, 8425). The multiple signals at 2900, 5700, and 8400 Da are due to removal of a hydroxybutyric acid end group during synthesis;[28] they are not caused by fragmentation in the mass spectrometer. No substantial fragmentation has been observed yet, using the MALDI-MS. Even very labile biological molecules are detected as intact ions.[30]

Obviously the PHB's with masses up to 8400 Da may be detected without problems (Figure 1). No analysis of larger derivatives than the 96mer has been carried out yet

Table I. Comparison of the Molar Ratios Oligomer/32mer Known from Weighing (Experimental Section; 1st and 2nd Determinations) and Analysis by GPC or MALDI-MS

	composn[b]		MALDI-MS[b]		GPC[b]	
oligomer	1st deter.[c]	2nd deter.	1st deter.	2nd deter.[a]	1st deter.	2nd deter.
16-mer	1.89	0.57	0.88	0.35	1.55	0.52
32-mer	1	1	1	1	1	1
64-mer	0.51	0.13	0.51		0.46	0.12
96-mer	0.35	0.04	0.31		0.27	0.04

[a] In the mass spectrum of the second mixture the 64mer and 96mer of narrow distribution are virtually undetectable; therefore, no evaluation was made. [b] Molar ratios of each oligomer to the 32-mer. [c] The observed deviations between the molar ratios determined by GPC and the initial composition are due to weighing errors (see caption of Figure 2).

but may be of importance for the determination of the molecular weight of the PHB constituent of the proposed ion channel.[29]

The corresponding GPC resolves the oligomer mixture into well-separated peaks (Figure 2); however, the loss of terminal groups is of course not detectable. As expected, there is a logarithmic relationship between molecular weight (more precisely the hydrodynamic volume) and the elution volume.

For mass resolutions ($m/\Delta m$ full width at half-maximum) between 300 and 600 the MALDI-MS always measures the average molecular weight of a given compound. All isotopic distributions become one single peak, and the centroid reflects the average mass.

However, the mass resolution in a time-of-flight MALDI instrument is not constant; there is always a systematic peak broadening with increasing mass. The reason for this behavior is (a) the vacuum pressure, (b) the laser intensity, and (c) the conversion efficiency for higher masses on the detector.[31] For evaluation of the correct mass proportions for low and high masses, the intensities have to be corrected. By comparison of the full width at half-maximum of different mass ranges and with different compounds, an empirical factor as a function of mass to correct the intensities of higher mass units was found. Multiplying every data point (offset corrected) with the appropriate calculated factor scales all peak intensities to those which would be obtained with a constant mass resolution. It should be noted that this algorithm corrects only the most important physical peak broadening effects as (a) and (b). Theoretically it is also possible to calculate the empirical factor with comparison of the peak areas at

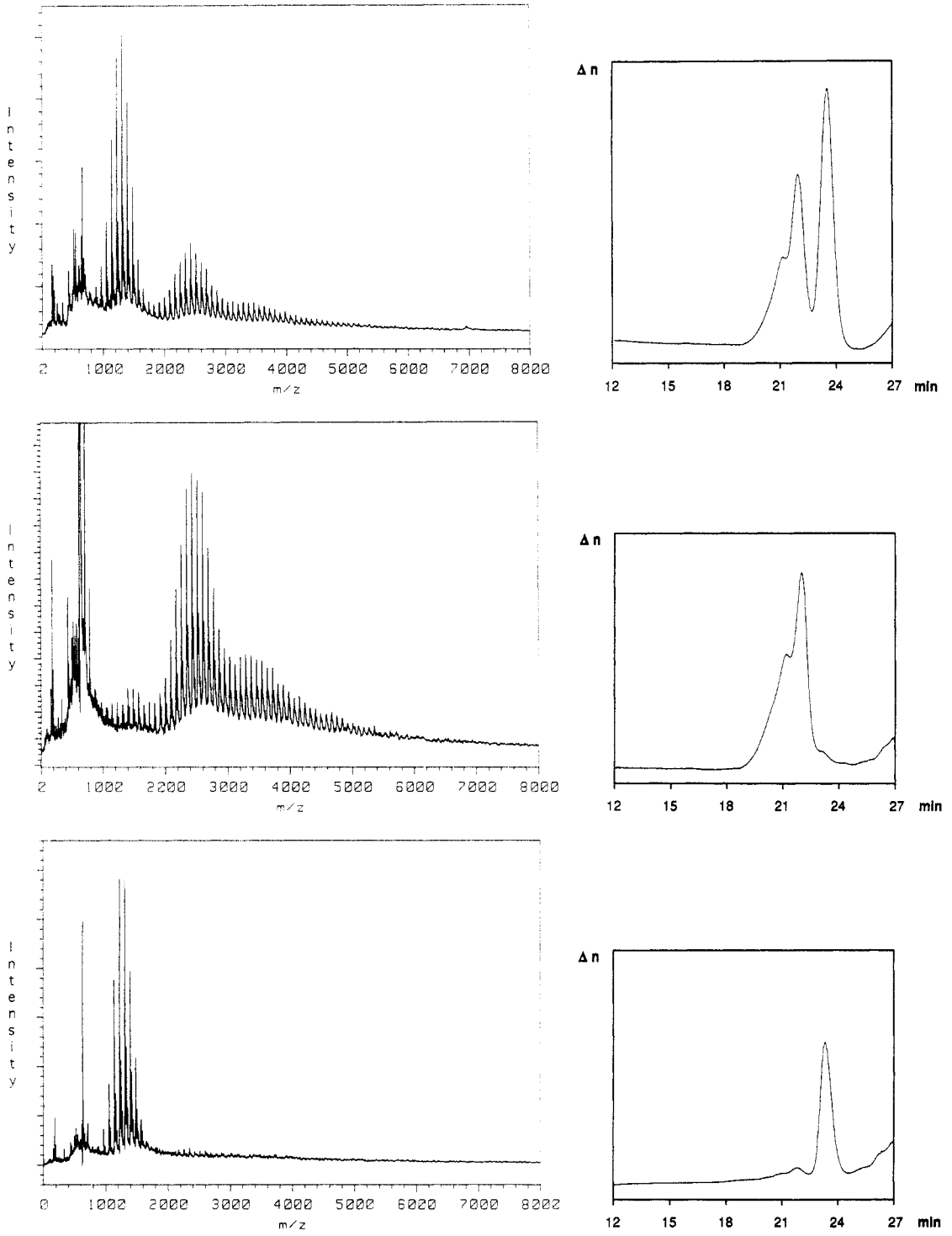


Figure 3. (a) Top: Positive ion MALDI-MS and GPC of the product **B1** obtained by three consecutive depolymerizations with LiHMDS. (b) Middle: MALDI-MS and GPC of the high molecular weight fraction (m > 1746 Da). (c) Bottom: Low molecular weight fraction (m < 1746 Da) separated by prepative gel chromatography on Sephadex LH 60. (Δn: Refractive index difference between sample and reference.)

different mass ranges. But with decreasing mass resolution the small additional peak of sodium and potassium adducts cannot be resolved any more. So these peak areas are too large, and now this kind of calculation leads to erroneous results.

In Figure 1 the measured mass peaks were therefore converted to signal intensities which one would get with constant mass resolution. The mass ratios determined for the oligomers, with the exception of the 16mer, showed a good correlation with those expected from the composition of the mixture (Table I). In contrast, the refractometric detection (Δn: refractive index difference between sample and reference) in GPC gives a signal which is proportional to the masses employed. Because of the identical number of monomeric units per mass unit the area under the peaks should be identical if a mixture of equal masses of oligomers is used. This was what we observed. Influences of the end groups may be ignored since they were insignificant considering the chain lengths of the oligomers studied (Table I).

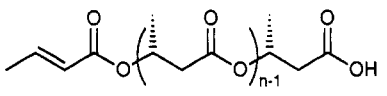
The higher oligomers (32mer, 64mer, 96mer) are detected in proportion to their abundance with both methods (Table I). The 16mer (m = 1540 Da) was detected by MALDI-MS with a sensitivity decreased by a factor of 1.7 as compared with GPC (average factor of both determinations in Table I; first determination, 1.8; second

determination 1.5). This mass spectrometric discrimination against the smaller masses disappeared at higher molecular weights and was already insignificant for the 32mer (m = 2918 Da). Nevertheless it will be shown in the following section that oligomer distributions may be quantified by MALDI-MS.

Partial Depolymerization of PHB with Lithium Amide Bases. The reaction products of the partial depolymerization of PHB with lithium hexamethyldisilazide/LiCl in THF at low temperature show a trimodal distribution (maxima 1600, 2700, 4200 Da) in the PD-MS.[11]

Quite surprisingly, there is only a small shift to smaller masses if the reaction products are treated repetitively under the specified conditions. Also, the yield of recovered and isolated oligomers is more or less constant in each repeated step (around 75%) and no complete degradation to crotonate is observed. The PHB derivatives obtained bear a crotyl ester group at the alcohol terminus. The mechanism of this reaction is still under investigation but seems not to proceed via a polyenolate as initially proposed.[11,12]

Figure 3a shows the MS and the GPC results using the oligomer mixture (type **B**) obtained by three successive



B

B1 $\overline{X}_n$ = 20 (Degradation with LiHMDS)

B2 $\overline{X}_n$ = 20 (Pyrolysis)

B3 $\overline{X}_n$ = 60 (Pyrolysis)

treatments of PHB with the lithium amide base. The oligomers in the region of 1000–3000 Da appear in the MS as clearly separated $[M + H]^+$ signals. The difference of m = 86 Da equals the mass of a monomeric unit ($C_4H_6O_2$). The smaller signals with m = 22 Da higher than the major peaks are due to $[M + Na]^+$ ions. Because of the decreasing resolution with increasing molecular weight the separation into discrete signals worsens for the higher molecular weights. Furthermore the intensities of the $[M + Na]^+$ signals increase in comparison with the $[M + H]^+$ signals, probably due to increased complexing tendencies of the longer chains with the cation.

Prior to analytical gel permeation chromatography, the free carboxy end groups of the oligomers (type **B**) were methylated with diazomethane, to avoid adsorption effects due to the polar end groups. The average chain length ($\bar{X}_n$) of this sample was also determined from its ^{1}H-NMR spectrum as the ratio $\sum C\text{-}CH_3/O\text{-}CH_3$ = 20.

The different appearance of the distributions in the MALDI-MS and the GPC in Figure 3a is due to (a) the already mentioned difference in the detected units (number of molecules in the MS *vs* mass of the molecules in the GPC), (b) the different abscissas (the retention time is proportional to the logarithm of the molecular weight in GPC), and (c) molecules with higher molecular weight eluting first in the GPC.

The evaluation of the MALDI-MS was performed as described above. The height of lines in Figure 4 equals the sum of peaks for the $[M + H]^+$ and $[M + Na]^+$ signals. Obviously the trimodal distribution is more apparent after the corrections were applied. Both methods yield essentially the same average molecular weights (Table II). The separate evaluation of the high (m > 1746 Da) and the low (m < 1746 Da) molecular weight parts of the spectra in

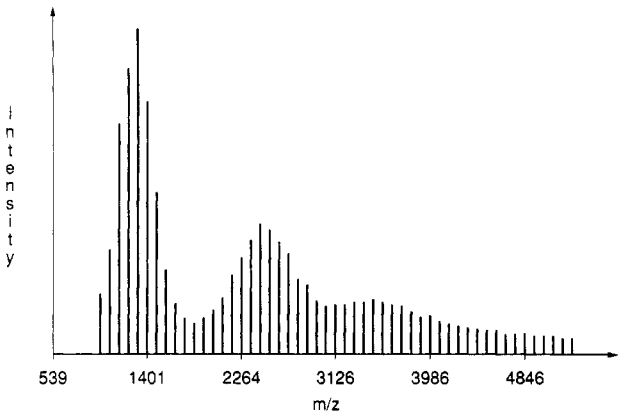


Figure 4. Line spectrum of the oligomers **B1**, obtained by correction of the original spectrum (Figure 3a) as described in the text. The factor of 1.7 was not employed.

Table II. Average Molecular Weights ($\bar{M}_w$, $\bar{M}_n$, $\bar{X}_n$, $\bar{M}_w/\bar{M}_n$) of the Oligomer Mixture of Type B1 Obtained by Three Repetitively Performed Depolymerizations of PHB with LiHMDS

method	fraction[a]	$\bar{M}_w$	$\bar{M}_n$	$\bar{X}_n$	$\bar{M}_w/\bar{M}_n$	mass proportion (high/low)
MS	both high and low					
	without factor	2837	2320	27	1.23	3
	with factor of 1.7	2608	2068	24	1.26	1.8
	high	3343	3105	36	1.08	
	low	1334	1310	15	1.02	
GPC	both high and low	2343	1816	21	1.29	1.1
	high	3288	2941	34	1.12	
	low	1310	1280	15	1.02	
isolation[b]						1.2

[a] The masses higher than m = 1746 Da are called high molecular weight fraction; all smaller ones are called the low molecular weight fraction. [b] From 150 mg of product **B1**, 74 mg of a high molecular weight fraction and 63 mg of a low molecular weight fraction were obtained; furthermore, 11 mg of a mixture containing equal amounts of both fractions (GPC) were isolated.

Figure 3a gives an even better correlation of $\bar{M}_w$ and $\bar{M}_n$ (Table II). There is, however, a major difference in the mass proportions calculated from the GPC and the MS data in Figure 3a. Depending on whether the previously calculated factor of 1.7 for the MALDI-MS (determined in Table I) is applied or not, mass ratios of 1.8 to 1 (the corrected signals of the low molecular weight fraction up to m = 1746 Da were multiplied by 1.7; the signals for the higher masses were not adjusted) or 3 to 1 were obtained. According to the GPC measurements the proportion of the high to the low molecular weight part is 1.1 to 1.

We were able to separate 150 mg of the oligomeric mixture **B1** on Sephadex LH60 into its major two parts. This preparative separation allowed us to analyze both fractions (for the definition of the fractions, see the caption of Table II) separately with MALDI-MS and GPC (Figure 3b) and, furthermore, gave the gravimetric proportion, which was 1.2 to 1. Therefore the factor seems to be less important for the characterization of oligomer distributions. However for determinations of mass ratios, it should be taken into account. The separation shows also that the low molecular weight part is not due to fragmentation in the MS or artifacts in the GPC.

Analysis of PHB Degraded by Pyrolysis. Heating of PHB to temperatures exceeding 175 °C leads to degradation yielding mixtures of type **B** oligomers; the average molecular weight is a function of the temperature and the duration of the pyrolysis. The cleavage occurs statistically within the chains, by ester pyrolysis.[10] Two samples **B2** ($\bar{X}_n$ = 20; 3 h at 210 °C) and **B3** ($\bar{X}_n$ = 60; 2

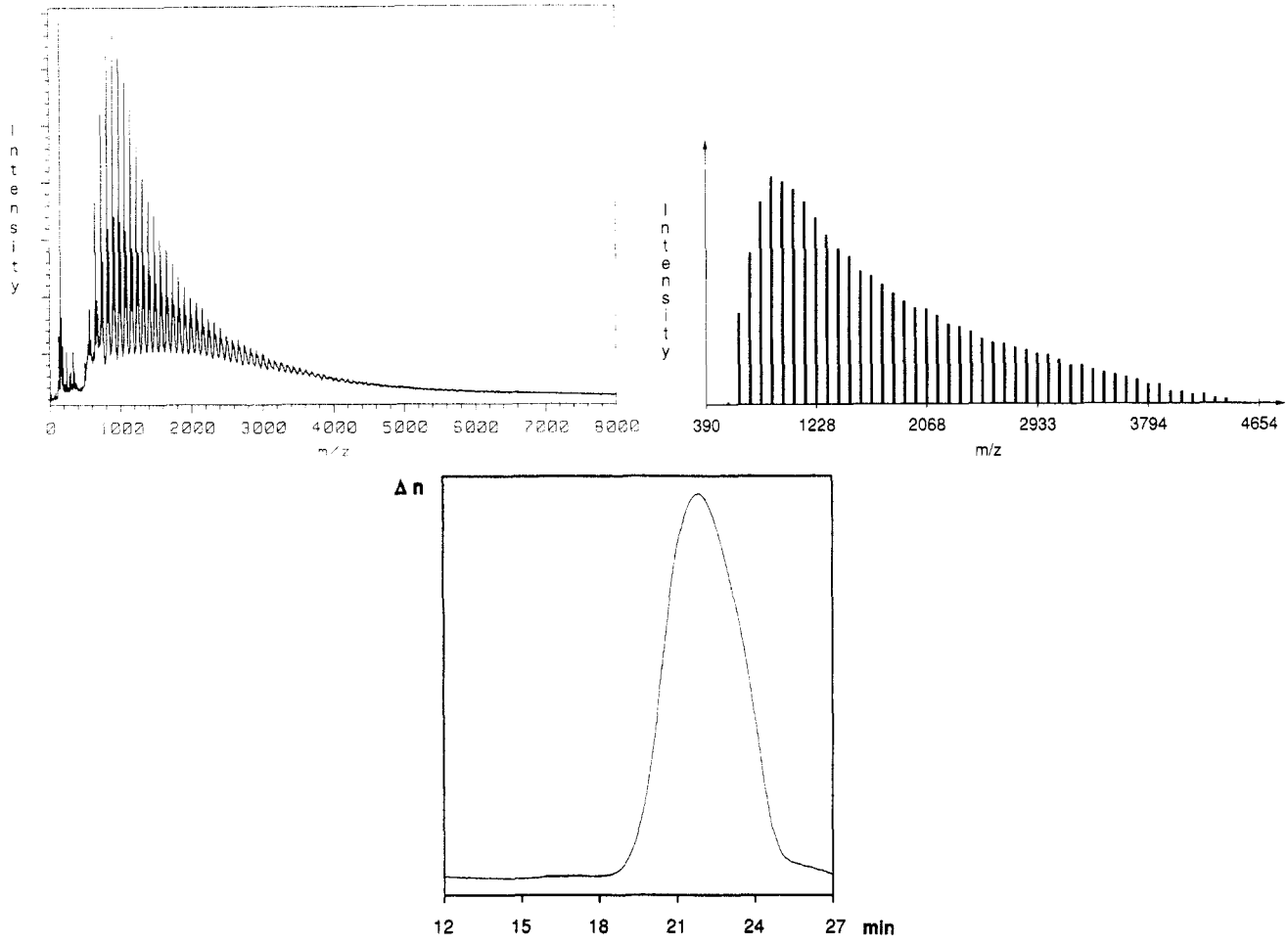


Figure 5. (a) Top: Positive ion MALDI-MS (left) and calculated line spectrum (right) of the oligomers **B2** obtained by pyrolysis of PHB at 210 °C for 3 h. (b) Bottom: Corresponding GPC. (Δn: Refractive index difference between sample and reference).

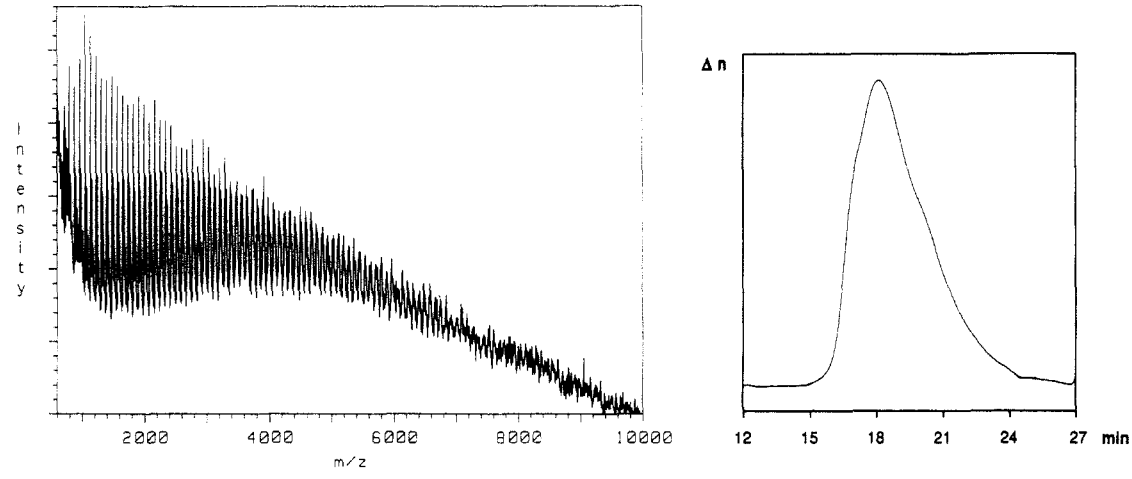


Figure 6. Positive ion MALDI-MS (left) and corresponding GPC (right) of the pyrolytically degraded PHB sample **B3**.

h at 210 °C) were analyzed using MALDI-MS and GPC (Figures 5 and 6). The number average degrees of polymerization $\bar{X}_n$ were determined by end group analysis as described in the Experimental Section. Again the peaks from the oligomers appeared as clearly separated $[M + H]^+$ signals; the smaller signals with a 22 Da higher mass are the corresponding $[M + Na]^+$ signals. The line heights in Figure 5a again equal the sum of the corrected areas and the $[M + H]^+$ and $[M + Na]^+$ signals.

For the relatively narrow distribution **B2** with $\bar{X}_n = 20$ a very good correlation was found for the average molecular weights determined by MALDI-MS and GPC; application of the correction factor 1.7 was not necessary (Table III). The limits of the MALDI-MS methods are reached with broadly distributed oligomers, such as the sample **B3** ($\bar{X}_n = 60$). The signals differ only slightly from the baseline (Figure 6), at least for the higher molecular weight part

Table III. Average Molecular Weights ($\bar{M}_w$, $\bar{M}_n$, $\bar{X}_n$, $\bar{M}_w/\bar{M}_n$) of the Oligomers of Type B Obtained by Pyrolysis

sample[a]	method	$\bar{M}_w$	$\bar{M}_n$	$\bar{X}_n$	$\bar{M}_w/\bar{M}_n$
B2	MS	2142	1724	20	1.24
	GPC	2285	1725	20	1.32
B3	MS[b]				
	GPC	10018	5770	67	1.74

[a] The number average degree of polymerization was $\bar{X}_n = 20$ for sample **B2** and $\bar{X}_n = 60$ for sample **B3**, as determined by end group analysis. [b] No evaluation was performed for reasons mentioned in the text.

of the spectrum, and a proper evaluation is therefore impossible.

Discussion

With the described matrix-assisted laser desorption and ionization MS another mass spectrometric method besides FAB- and PD-MS has been shown to be very useful for the analysis of oligomers of (*R*)-3-hydroxybutanoic acid. In contrast to both of the other techniques MALDI-MS permits detection of these oligomers of masses up to m = 8500 Da (96mer in Figure 1), and probably even larger oligomers might be detected. After taking the peak areas and the systematic loss of resolution into account, the 16mer (m = 1540 Da) is detected less sensitively—by a factor of 1.7—than the compounds with masses larger than 2800 Da. However the 32mer (m = 2918 Da), the 64mer (m = 5677 Da), and the 96mer (m = 8425 Da) are then detected in proportion to their molar abundance.

As expected, GPC is very reliable for the analysis of hydroxybutyric acid oligomers. The refractive index detector gives a signal proportional to the mass of the compounds, and no influences from the end groups of the compounds (type **A**) were detectable (Table I). Both methods are useful for characterizing distributions of type **B** oligomers, although the GPC method is more reliable and useful for a broader range of applications. Nevertheless rather complex distributions, like the one obtained by three consecutive treatments with LiHMDS, give satisfying results with MALDI-MS (Figure 3a). From quantitation experiments on peptides,[32] it is known that the compound with the predominant concentration in a given mixture will appear in the MALDI spectra as the highest peak but against the other byproducts it will be underrepresented. The present data show similar effects.

The correlation between the average molecular weights determined by MALDI-MS and GPC is better the narrower the distribution of the sample is (Tables II and III). An even better correlation is found when the empirical factor of 1.7 is used for the evaluation of the mass spectra (Table II), although it is necessary to calibrate the spectrometer with defined oligomers for determining this factor. Up to now oligomers of type **A** are not commercially available (and their synthesis is elaborate), and gel permeation chromatography is the method of choice for the analysis of broad distributions.

To our knowledge, the MALDI-MS is the best method known for mass spectrometric analysis of poly[(*R*)-3-hydroxybutanoates] and its derivatives. We have reported here the first determinations of PHB species with the MALDI method. Further optimization of the hardware and a deeper knowledge of the processes occurring will allow even more accurate determinations.

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