Electrospray ionisation FT-ICR mass spectrometry of linear and hyperbranched polymers
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Chapter 8

Isomer separation of hyperbranched polyester-amides with gas phase H/D exchange and a novel MS$^n$ approach: DoDIP

Two approaches are introduced that provide information about the isomeric composition of hyperbranched polyester-amides. The first approach is based on a novel MS$^n$ approach that allows studying different types of isomeric structures by a separation based on their difference in appearance energy. The method is called DoDIP: Dissociation of Depleted Ion Populations. A first MS/MS step is used to fragment isomers with relatively low appearance energy. The isomers with higher appearance energy are fragmented in a second MS/MS step.

The second approach is based on gas phase H/D exchange experiments that result in a bimodal isotopic distribution for oligomers $X_n D_{n+1}$ of which one distribution corresponds to a type of isomeric structure that exhibit H/D exchange behaviour and the other one to an isomeric structure that does not exhibit H/D exchange behaviour. $X$ is a difunctional anhydride of phthalic acid, 1,2-cyclohexane dicarboxylic acid, succinic acid, or glutaric acid, D is a trifunctional diisopropanolamine and n the degree of polymerisation. The type of isomeric structure that does not exhibit H/D exchange behaviour has a non-alternating monomer sequence that contains an amine bond with a relatively high proton affinity. The other isomeric structure that does exhibit H/D exchange behaviour has an alternating monomer sequence containing only amide and ester bonds with relatively low proton affinity. Oligomer structures were confirmed with additional MS$^2$ experiments after H/D exchange. H/D exchange experiments on the fragments obtained after MS$^2$ of the parent ion disprove two postulated mechanisms (mechanisms 7.2 and 7.3) for the cleavage of the ester and amide bond. A new mechanism is introduced to explain the H/D exchange behaviour of the fragments that requires a cleavage of the amide bonds only. Two types of fragments are formed by this mechanism. One type is protonated due to the cleavage of the amide bond whereas the other type has an oxazolonium ion structure due to the loss of an additional $H_2O$. 
8.1. Introduction

Ion-molecule reactions have long been used to obtain detailed structural information of organic compounds. The first studies involved small molecules like amino acids. Studies of large molecules have become popular in the last decade after the soft ionisation techniques electrospray ionisation (ESI) and matrix-assisted laser desorption/ionisation (MALDI) were introduced. These ionisation techniques allow the transfer of large molecules intact into the gas phase to study their chemical structure. Most ion-molecule reactions reported on large molecules concern biomolecules. Isomer identification poses a special problem for mass spectrometry as isomers have the same \( m/z \) value. Isomeric structures of small molecules, for example xylenes, can be distinguished with hydrogen/deuterium (H/D) exchange experiments due to a difference in the number of exchangeable hydrogens and exchange rate. For larger molecules, mainly biomolecules, different rates of deuterium incorporation have also been observed. This is however not a result of differences in isomeric structures but caused by differences in the gas phase conformation of the biomolecules. Different conformations have a different proton accessibility along the chain responsible for the H/D exchange. Other important factors in the reaction kinetics of deuterium incorporation during the H/D exchange events are the proximity of functional groups, differences in the proton affinity between analyte and reagent gas and temperature.

Beauchamp and coworkers proposed the relay mechanism for the H/D exchange activity of biomolecules. This mechanism requires a proton transfer from the site of protonation in the hydrogen bonded complex to gas phase D\(_2\)O. At the same time, a deuteron is transferred from D\(_2\)O to a distant, slightly less basic site on the molecule. A nice feature of this mechanism is that only protonated ions exhibit H/D exchange activity. This phenomenon provides a tool to study fragmentation mechanisms that may lead to non-protonated ions.

The hyperbranched polymers studied in this chapter are made by the polycondensation of a trifunctional (D) and a difunctional (X) monomer (see chapter 7). Alternating oligomer sequences with amide and ester bonds only are ideally formed during the polymerisation reaction leading to mainly oligomer series X\(_n\)D\(_{n+1}\) because D was added in access to the polymerisation. Such oligomers contain OH endgroups. Oligomer series X\(_n\)D\(_{n+1}\) can also consist of a non-alternating sequence, although this is less likely, when two D monomers are connected by an amine. Such oligomers contain at least one carboxylic acid endgroup, which might influence the cross-linking properties of these polymers when applied as cross-linkers in powder coatings. It is therefore important to determine which type of oligomer is present. Note that when doing a single mass
spectrometric analysis of such polymers the alternating and non-alternating monomer sequences cannot be distinguished because they are isomeric structures. We describe here two approaches that allow the distinction between isomeric structures of hyperbranched polyesteramides. The first approach is a novel MS\textsuperscript{n} methodology using a two-step collisionally activated dissociation (CAD) process called Dissociation of Depleted Ion Populations (DoDIP). The second approach is based on gas phase H/D exchange. CAD is a widely used technique to obtain structural information of biomolecules and synthetic polymers. To the knowledge of the authors, dissociation techniques have never been used to determine whether the oligomers in synthetic polymers consist of mixture of different isomers.

8.2. Experimental

The analyses were performed with a modified FT-ICR MS (Bruker-Spectrospin APEX 7.0e, Fällanden, Switzerland). The cell was an in house constructed open cell. Pulsed gas trapping with Argon at P\textsubscript{Ar}=5.2\cdot10^{-6} mbar for 2 seconds was used to enhance trapping of the ions in the open cell. On-resonance excitation collisionally activated dissociation in the ICR cell was used to activate the parent ions generated by electrospray ionisation. Argon was used as collision gas (P\textsubscript{Ar}=5.2\cdot10^{-6} mbar). The geometry factor $\alpha$ of the open cell necessary for the calculation of the excitation potentials on the excitation electrodes was 2.26. The peak-to-peak voltage of the RF excitation signal was 17.6 V in all CAD experiments. For the energy resolved experiments, three scans were summed at each collision energy. H/D exchange experiments were performed by pulsing D\textsubscript{2}O in the ICR cell using a pulsed needle valve. See chapter 2 for a more detailed description of the experimental set-up.

The hyperbranched polyesteramides used for this study are polycondensation products of the trifunctional di-isopropanolamine (D) and a difunctional dicarboxylic acid anhydride provided by DSM (Geleen, the Netherlands).\textsuperscript{231} The difunctional dicarboxylic acid anhydrides used in the polymerisations are succinic acid anhydride (S), glutaric acid anhydride (G), phthalic acid anhydride (P) and 1,2-cyclohexane dicarboxylic acid anhydride (C). Figure 7.1 shows the structures of the monomers.
8.3. Results and discussion

The mass spectrum of the phthalic acid anhydride based polymer with an average molecular weight of 1000 Da is presented in figure 8.1. The spectrum reveals the presence of a series of protonated and sodiated oligomers that were observed earlier in FD, MALDI and ESI studies of these polymers. The most intense oligomer series, $P_n D_{n+1}$, contains di-isopropanolamine (D) endgroups and are all expected to have an alternating structure. The $n$ denotes the number of monomers in a given oligomer. Cyclic alternating oligomers $P_n D_n$ were also observed. The desired oligomer series $P_n D_{n+1}$ is formed by the reaction of an oxazolonium functionality with an OH endgroup (pathway A in figure 7.3). Oligomer series $P_n D_{n+2}$ and $P_n D_{n+3}$ are formed by the reaction of the oxazolonium ion with an amine that is also present in the polymerisation mixture (pathway B in figure 7.3). The polymerisation conditions were chosen such that the concentration of amine groups during the polymerisation is low and the relative amount of $P_n D_{n+2}$ and $P_n D_{n+3}$ in the final polymer is lower than $P_n D_{n+1}$.

![Figure 8.1](image)

Figure 8.1  ESI FT-ICR MS spectrum of hyperbranched polyesteramides based on phthalic acid anhydride and di-isopropanolamine with an average molecular weight of 1000 Da.

The fragmentation behaviour of the hyperbranched polyesteramides synthesised with the anhydrides of 1,2-cyclohexane dicarboxylic acid, phthalic acid, succinic acid and glutaric acid has been discussed in chapter 7. However,
these MS\(^2\) and MS\(^3\) studies did not lead to an understanding of the isomeric composition of the hyperbranched polymers. Oligomer \([P_3D_4+H]^+\) with \(m/z = 923.46\) can have, for example, several isomeric structures, of which four examples are shown in figure 8.2. The P\(_3\)D\(_4\) oligomer can have an alternating branched structure (I) although alternating linear structures (II and III) are possible as well. Note that the difference between structures II and III is the inverted orientation of one of the di-isopropanolamine groups. Structure III is symmetrical whereas structure II is not. Although the structures are very different, they all contain OH endgroup functionalities only. In principle, P\(_3\)D\(_4\) can also have an isomer with a non-alternating structure due to pathway B in figure 7.3 (structure IV). This has so far not been considered in the literature. Structure IV contains an amine functionality which is not present in the alternating structures. More important, structure IV contains one COOH endgroup functionality. It is important from a polymer chemistry point of view to know whether oligomer series with these functionalities is formed in the polymerisation process.

8.3.1. Dissociation of Depleted Ion Populations (DoDIP) for isomer analysis

The MS/MS spectrum of \([P_3D_4+H]^+\) resulting from on-resonance CAD with Argon and a laboratory frame kinetic energy \(E_{\text{kin,lab}} = 129\) eV is presented in figure 8.3a. Many fragments are formed but it is not possible to retrieve the presence of different isomeric structures of P\(_3\)D\(_4\), because the MS/MS behaviour of the pure isomers is not known. Fractionation prior to MS analysis has not been performed but could be an alternative way to obtain the MS/MS spectra of the pure isomers. Fractionation would however be very difficult due to the high number of possible linear, branched, alternating and non-alternating isomers. Reactions leading to the fragments in figure 8.3a will be discussed briefly based on the fragmentation pathways described in chapter 7, although results that will be described later demonstrate that the mechanisms for the cleavage of the ester and amide bonds (mechanisms 7.2 and 7.3) are not correct. For reasons of clarity, we will first use the ‘old’ notation for the fragments as introduced in chapter 7 and demonstrate later that exclusively amide bonds are rearranged for which we will propose another mechanism.
Figure 8.2 Four possible isomeric structures of oligomer $P_3D_4$. The first three structures are alternating oligomers whereas the fourth is non-alternating (two di-isopropanolamines connected by an amine bond, see circle).

Fragment $[P_3D_4-H_2O]^{ox+}$ has been proposed to be due to the loss of $H_2O$ from one of the OH endgroups (mechanism 7.1) forming oxazolonium ions denoted by the superscript $^{ox+}$. Ester and amide bond cleavages also lead to oxazolonium ions $^e[PD_2-H_2O]^{ox+}$, $^e[P_2D_3-H_2O]^{ox+}$, $^b[PD-H_2O]^{ox+}$, $^b[P_2D_2-H_2O]^{ox+}$ and $^b[P_3D_3-H_2O]^{ox+}$, in which the superscripts $^e$ and $^b$ correspond to an ester and amide cleavage, respectively. An additional $H_2O$ loss is observed leading to $^e[PD_2-2H_2O]^{ox+}$, $^e[P_2D_3-2H_2O]^{ox+}$, $^b[PD-2H_2O]^{ox+}$, $^b[P_2D_2-2H_2O]^{ox+}$ and
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$^{5}[P_3D_3-2H_2O]^{ox^+}$. These fragments have been explained by an ester or amide bond rearrangement followed by the loss of H$_2$O. Finally, fragment $^{7}[P_3D_4+H]^+$, due to a $\gamma$-Hydrogen rearrangement, is observed.

![Diagram](image)

**Figure 8.3** $MS^2$ spectrum of $[P_3D_4+H]^+$ with $E_{kin,lab}= 129$ eV (a) DoDIP mass spectrum of $[P_3D_4+H]^+$ with $E_{kin,lab}= 70$ eV (~75% survival yield) for $MS^2$ followed by $E_{kin,lab}= 129$ eV for the second MS/MS step (b) DoDIP mass spectrum of $[P_3D_4+H]^+$ with $E_{kin,lab}= 129$ eV (~30% survival yield) for $MS^2$ followed by 129 eV collision energy for the second MS/MS step (c).
The survival yield of the ions is plotted in the breakdown diagram as a function of the collision energy. The survival yield of the parent ion is defined as the intensity of the parent ion divided by the sum of the intensities of the parent ion and all fragments. To obtain the survival yield of the fragments, the intensity of a particular fragment is divided by the sum of the intensity of the parent ion and all fragment ions. Several fragmentation pathways are distinguished from the breakdown diagram of [P_3D_4+H]^+ (figure 8.4). A loss of H_2O had the lowest appearance energy followed by the rearrangement of the ester and amide bonds as discussed in chapter 7. Rearrangement of the ester and amide bond with an additional loss of H_2O to allylic or morpholine endgroups had the highest appearance energy. For reasons of clarity, only 4 of the 13 fragments that were observed and the parent ion are plotted in figure 8.4. All fragments have been included in the calculation of the breakdown diagram, however.

![Breakdown diagram of [P_3D_4+H]^+](image)

**Figure 8.4** Breakdown diagram of [P_3D_4+H]^+. Only four fragments are shown in the figure with × [P_3D_4-H_2O]^+ox, ⋅⋅⋅ [P_2D_3]^+ox, ⋅ [P_2D_2-H_2O]^+ox, ⋅⋅⋅ (PD)^+ox and parent ion + [P_3D_4]^+H^+.

The breakdown diagram of [P_3D_4+H]^+ shows that the intensity of the different fragments changes with the collision energy (figure 8.4) resulting in a different composition of the MS/MS spectra with the collision energy. This effect can be explained by different appearance energies for the different fragmentation pathways of the parent ion. On the other hand, the effect may be caused by different isomeric structures that start fragmenting at different appearance energies.
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The reconstructed breakdown diagram does not allow distinguishing between the isomers because the MS/MS spectrum at each collision energy is a summation of all fragments of all isomers.

A way to determine whether P3D4 contains different isomeric structures that have different appearance energies for fragmentation is by performing a novel CAD experiment on parent ion [P3D4+H]+ where depleted ion populations are fragmented. Approximately 25% of the parent ion [P3D4+H]+ population is fragmented in the first MS/MS step (to ~75% survival yield see figure 8.4) with \( E_{\text{kin,lab}} \) of 70 eV. After a thermalisation delay with an Argon gas pulse of 5 seconds, the remaining ~75% of [P3D4+H]+ is isolated and fragmented with \( E_{\text{kin,lab}} = 129 \) eV. This thermalisation delay is required to cool down the ion population to room temperature and release the kinetic energy and coherence of the remaining parent ions. Note that this is not a regular MS\(^3\) experiment but a novel MS\(^n\) approach called Dissociation of Depleted Ion Populations (DoDIP). The spectrum that is obtained is presented in figure 8.3b. An experiment where the depleted ion population is fragmented using 70 eV has not been performed.

A similar DoDIP experiment is performed when ~70% of the parent ion population is fragmented (~30% survival yield) in the first MS/MS step with \( E_{\text{kin,lab}} = 129 \) eV. The DoDIP mass spectrum is shown in figure 8.3c. All other conditions are the same as the DoDIP experiment described in figure 8.3b. The basic idea behind this experiment is that isomers with relatively high appearance energy for fragmentation have not fragmented in the first low energy MS/MS step. The relative abundance of the isomeric ions that have relatively high appearance energy for fragmentation is increased before fragmentation in the DoDIP experiment at higher collision energy. Comparing the two DoDIP mass spectra provides information about isomeric structures if any are present. Note that this procedure relies on isomers that have a relatively large difference in their appearance energy.

All fragments observed in figure 8.3c are also observed in figure 8.3b. This is because the first MS/MS step in figure 8.3b involves fragmentation to only ~75% survival yield but the appearance of the DoDIP mass spectrum could be due to the fragmentation of multiple isomeric structures. The DoDIP mass spectrum in figure 8.3c is most probably due to only one type of isomeric structure with relatively high appearance energy for fragmentation. Figures 8.3b and 8.3c clearly demonstrate that P3D4 consists of at least two isomeric structures or two types of isomeric structures.

The question arises which (type of) isomeric structures are responsible for the differences in the DoDIP mass spectra. One possibility is that the polymer consists of a branched structure and a linear structure. However, similar results as
shown in figure 8.3 were obtained when parent ion \([P_2D_3+H]^+\) was studied with DoDIP experiments. This oligomer is too small to have a branched structure. The difference in figures 8.3b and 8.3c can therefore not be attributed to branching. Another possibility is that one (type of) isomeric structure is alternating like I, II and/or III (figure 8.2) and the other is non-alternating like IV. To test whether one of the isomers has a non-alternating sequence, an ion with a non-alternating structure, \([P_2D_4+H]^+\), is fragmented. The MS/MS spectrum of ion \([P_2D_4+H]^+\) with \(E_{\text{kin}, \text{lab}} = 154\) eV is shown in figure 8.5.

Fragments \(b[P_2D_2]^{ox+}\) and \(b[PD]^{ox+}\) were expected to appear in figure 8.5, based on fragmentation studies discussed in chapter 7, but are not observed. Also, the loss of \(H_2O\) to \([P_2D_4-H_2O]^{ox+}\) and a \(\gamma\)-hydrogen rearrangement leading to \(\gamma[P_2D_3+H]^+\) are absent. The two most intense fragments that are observed, \(c[PD_2-H_2O]^{ox+}\) and \(c[P_2D_3-H_2O]^{ox+}\), are explained by ester rearrangements (mechanism 7.3). However, these fragments can also originate from cleavages of the amine bonds making the interpretation of the MS\(^2\) spectra more complex. The intensity profile of the fragments did not change dramatically when the collision energy was varied indicating that probably only one (type of) isomeric structure is present (breakdown diagram not shown).

![Figure 8.5](MS/MS spectrum of non-alternating oligomer \([P_2D_4+H]^+\) with a collision energy of 154 eV.)
The DoDIP behaviour of the P\textsubscript{3}D\textsubscript{4} isomer in figure 8.3c shows similar fragmentation behaviour as [P\textsubscript{2}D\textsubscript{4}+H]\textsuperscript{+}. However, the DoDIP behaviour of the P\textsubscript{3}D\textsubscript{4} isomer in figure 8.3c does not result in fragment [PD\textsubscript{3}-H\textsubscript{2}O]\textsuperscript{ox+}, which is observed upon fragmentation of [P\textsubscript{2}D\textsubscript{4}+H]\textsuperscript{+} and is a sequence specific fragment for non-alternating sequences. The intensity of this fragment was however low for the MS/MS of [P\textsubscript{2}D\textsubscript{4}+H]\textsuperscript{+} shown in figure 8.5. All fragments of the DoDIP of [P\textsubscript{3}D\textsubscript{4}+H]\textsuperscript{+} in figure 8.3c are of low intensity due to a reduction of the precursor ion in the first MS/MS step to 30\% survival yield. The intensity of [PD\textsubscript{3}-H\textsubscript{2}O]\textsuperscript{ox+} is therefore probably below the detection limit. The MS/MS spectrum of [P\textsubscript{3}D\textsubscript{4}+H]\textsuperscript{+} does also not show the loss of H\textsubscript{2}O, which is observed for the MS/MS of [P\textsubscript{3}D\textsubscript{4}+H]\textsuperscript{+} shown in figure 8.3c but the intensity profile of the other fragments of [P\textsubscript{2}D\textsubscript{4}+H]\textsuperscript{+} is very similar to the intensity profile of the fragments of [P\textsubscript{3}D\textsubscript{4}+H]\textsuperscript{+} in figure 8.3c.

The absence of fragment [P\textsubscript{2}D\textsubscript{3}+H]\textsuperscript{+} in both spectra (figure 8.3c and 8.5) and the similar fragment intensity profile both indicate that figure 8.3c is due to an isomer of P\textsubscript{3}D\textsubscript{4} with a non-alternating sequence. The non-alternating isomer of P\textsubscript{3}D\textsubscript{4} has at least one carboxylic acid endgroup, which might be the cause for the loss of one H\textsubscript{2}O. P\textsubscript{2}D\textsubscript{4} does not contain carboxylic acid endgroups.

A difference in the fragmentation behaviour can be accounted for by a difference in the proton affinity between the alternating and non-alternating structures. Absolute proton affinities (PA) of the molecules studied here have not been measured or reported in the literature but table 8.1 shows the literature values of the absolute proton affinities (PA in kJ/mol) of four small esters, three amides and three tertiary amines that have structural similarities with the molecules studied here. The tertiary amines have a proton affinity that is \(\sim 60\) kJ/mol higher than the amides and \(\sim 135\) kJ/mol higher than the esters. It is assumed that a similar behaviour of the PA will occur for the molecules studied here. The non-alternating structure of P\textsubscript{3}D\textsubscript{4} contains a tertiary amine, which has a higher proton affinity than an amide or ester bond. Therefore the proton will be more strongly bound to the tertiary amine bond of P\textsubscript{3}D\textsubscript{4} and non-alternating P\textsubscript{3}D\textsubscript{4}. If the proton is involved in the fragmentation of the parent ion, a higher collision energy will be required to fragment isomers with a relatively high PA.

A hydrogen rearrangement leading to fragment \([P\textsubscript{2}D\textsubscript{3}+H]\textsuperscript{+}\) requires a higher internal energy than the other fragmentation pathways whereas in case of the alternating ion [P\textsubscript{3}D\textsubscript{4}+H]\textsuperscript{+}, the proton will be adjacent to the amide carbonyl oxygens and a hydrogen rearrangement requires a relatively low collision energy.
8.3.2. Combined hydrogen/deuterium (H/D) exchange and MS² for isomer analysis

Gas phase hydrogen/deuterium (H/D) exchange experiments were performed with the entire polymer MWD without isolation of a specific ion to determine in one analysis, which of the ions exhibit H/D exchange activity. The D₂O background pressure was 5·10⁻⁷ mbar for 120 seconds. Some of the observations are summarised in figure 8.6 where the original isotopic patterns of the parent ion (due to ¹³C, ²H, ¹⁵N and ¹⁸O) are compared with the isotopic patterns after H/D exchange. Figure 8.6 demonstrates that only protonated species exhibit H/D exchange activity. Sodiated species, like [P₃D₄+Na]⁺, are present in the spectra but have not been observed to exchange hydrogen with deuterium. This is in agreement with the relay mechanism that was introduced to explain the H/D exchange mechanism. The relay mechanism requires a mobile proton for H/D exchange.

<table>
<thead>
<tr>
<th>Proton affinities in kJ/mol</th>
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<tbody>
<tr>
<td>Esters</td>
</tr>
<tr>
<td>CH₃COOCH₃</td>
</tr>
<tr>
<td>C₆H₅COOCH₃</td>
</tr>
<tr>
<td>CH₃COOC₂H₅</td>
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<tr>
<td>C₆H₅COOCH₃</td>
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<tr>
<td>Amides</td>
</tr>
<tr>
<td>(CH₃)₂NCHO</td>
</tr>
<tr>
<td>(CH₃)₂NCOCH₃</td>
</tr>
<tr>
<td>C₆H₅CON(CH₃)₂</td>
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<tr>
<td>Tertiary amines</td>
</tr>
<tr>
<td>(CH₃)₂(C₆H₅)N</td>
</tr>
<tr>
<td>(CH₃)(C₆H₅)₂N</td>
</tr>
<tr>
<td>(C₆H₅)₃N</td>
</tr>
</tbody>
</table>

Table 8.1  Proton affinities (kJ/mol) of molecules with structural similarities to the hyperbranched polyesteramides studied in this work.

Ion series [PₙDₙ⁺+H]⁺ shows H/D exchange activity, see for example [P₃D₄+H]⁺ in figure 8.6, while the ion series that has a non-alternating monomer sequence, [PₙDₙ+2⁺+H]⁺, does not (for example [P₃D₅+H]⁺). Even after a longer exchange time of 30 min, no hydrogens of ion series [PₙDₙ+2⁺+H]⁺ were exchanged by deuterium (results not shown) although this ion series contains many exchangeable hydroxyl hydrogens. This phenomenon is explained by the difference in the proton affinity between structures PₙDₙ⁺+ and PₙDₙ+2. Oligomer
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series $P_nD_{n+1}$ consist of amide and ester bonds only, if the polymerisation conditions are optimal. Oligomer series $P_nD_{n+2}$ on the other hand consist of amide and ester bonds with an additional tertiary amine bond between two di-isopropanolamines. A tertiary amine has a higher proton affinity (see table 8.1) than an amide or ester bond as discussed in the previous section. Therefore the proton will be relatively strongly bound to the tertiary amine bond (proton is less mobile) of $[P_nD_{n+2}+H]^+$ making H/D exchange more difficult. In case of alternating ion series $[P_nD_{n+1}+H]^+$, the proton will most probably be mobile and adjacent to the amide carbonyl oxygens that have a relatively low PA and therefore exhibit a higher H/D exchange activity.

Ion series $P_nD_{n+2}$ does exhibit H/D exchange behaviour when the charge state is 2, see for example $[P_6D_8+2H]^{2+}$. This is explained by one proton being fixed at the tertiary amine whereas the other proton is mobile and responsible for the H/D exchange behaviour. Similar results as shown in figure 8.6 were obtained when mono-isotopic peaks were isolated prior to H/D exchange.

An interesting feature is the bimodal nature of the isotopic pattern of $[P_3D_4+H]^+$ after the H/D exchange event. This has been observed for all oligomers from the ion series $[P_nD_{n+1}+H]^+$. Several studies have shown that such bimodal distributions can be correlated to the secondary structure of the molecule (mostly biomolecules).\textsuperscript{246,248,250,253} For the molecules studied here, it could be possible that the two distributions are due to the presence of branched and non-branched oligomers. However, the isotopic pattern of oligomer $[P_2D_3+H]^+$ is also split into two distributions after the H/D exchange event (see figure 8.6), but this oligomer is too small to be branched. It is therefore unlikely that the two distribution of $[P_3D_4+H]^+$ are due to branched and linear structures.
Figure 8.6 *Isotopic profiles of some oligomers before (lower) and after (top) H/D exchange at 5·10⁻⁷ mbar D₂O for 120 seconds.*

Here, the two distributions are due to the presence of alternating and non-alternating isomeric structures. The ion series contain isomers that do exhibit H/D exchange behaviour and isomers that do not. Based on the observations discussed above, it is most likely that the [P₃D₄+H]⁺ isomer with H/D exchange behaviour has an alternating sequence with a relatively low PA like structures I, II or III in figure 8.2. The [P₃D₄+H]⁺ isomer that does not exhibit H/D exchange behaviour is a non-alternating oligomer resulting from an error during the polymerisation reaction, which contains a tertiary amine with a high proton affinity (structure IV). Additional MS² experiments were performed to confirm these structures. The H/D
exchange experiment was repeated after isolation of the first two isotopic peaks of $[P_3D_4+H]^+$ at 5-10^{-7} \text{ mbar} \text{ for} \text{ 2 minutes.} Two \text{ MS}^2 \text{ experiments were performed after the H/D exchange event;} 1) \text{ isolation of the first two isotopic peaks (non H/D exchanging) followed by CAD (figure 8.7a)} \text{ and 2) isolation of all isotopic peaks with more than 5D (\geq 5 \text{ H/D exchanges}) followed by CAD (figure 8.7b). Although all experimental conditions were the same, figures 8.7a and 8.7b clearly show differences. The most important differences are the absence of fragments $^a[P_2D_3+H]^+$ and $^b[P_3D_3-H_20]^+$ in figure 8.7a. This has been observed earlier in figure 8.3 for the DoDIP experiments. Figure 8.7a shows similarities with figure 8.3c, which is a result of a non-alternating sequence. Figure 8.7b shows similarities with figure 8.3b resulting from an alternating sequence. These results clearly demonstrate that alternating and non-alternating isomers can be separated using both gas phase H/D exchange and DoDIP experiments.

An experiment is performed to check whether the separation of the isomers by H/D exchange is complete. After the first H/D exchange event on the monoisotopic peak of $[P_3D_4+H]^+$ (results not shown), the monoisotopic peak (containing non-alternating $[P_3D_4+H]^+$) is isolated again followed by a second H/D exchange event of 2 minutes. No hydrogens were exchanged during the second H/D exchange event indicating that only non-alternating isomers were present in the ICR cell, or at least below the detection threshold.

If the H/D exchange of, for example, mono-isotopic $[P_3D_4+H]^+$ would be complete, only two peaks should be obtained: one for the alternating and one for the non-alternating isomers. The H/D experiments described above show, however, a broad (bimodal) distribution, which does not change much with an increasing H/D exchange reaction time. Several authors have described earlier that extensive backexchange can occur (deuterium exchanged by hydrogen). This is due to collisions of the deuterated agent with the wall of the ICR cell, which is saturated with hydrogen containing molecules, for example H$_2$O. Deuterium will be exchanged by hydrogen resulting in the gas phase molecules HDO and H$_2$O. A way to overcome this problem is by prior saturation of the cell wall with D$_2$O. These experiments have not been performed here, which is most probably the cause of the broad distributions observed.
Chapter 8

Figure 8.7  H/D exchange experiment on the first two isotopic peaks of parent ion \([P_3D_4+H]^+\) with \(D_2O\) for 120 seconds at \(5 \times 10^{-7}\) mbar. The experiment was followed by MS/MS after isolation of the first two isotopic peaks (a) and MS/MS after isolation of the isotopic peaks with more than \(5\) H/D exchanges (b).
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8.3.3. Combined MS\(^2\) and hydrogen/deuterium (H/D) exchange for the study of the fragmentation mechanism

Mechanisms 7.2 and 7.3 have been proposed that explain the fragmentation behaviour of the ester and amide bonds of hyperbranched polyesteramides.\(^9\) Both mechanisms lead to the formation of oxazolonium ions. An oxazolonium ion has, in contrast to a protonated fragment, a charge fixed on the molecule by a quaternary ammonium ion. Because it was not possible to confirm the mechanisms and structure of these ions, we performed an experiment to find out whether the fragments contain a 'mobile' proton or a fixed charge (oxazolonium ion), see also the addendum of chapter 7. The mono-isotopic parent ion \([P_3D_4+H]^+\) was isolated and fragmented using collisionally activated dissociation (\(E_{\text{kin,lab}}=185\) eV) followed by H/D exchange to establish if a mobile proton is present. All fragments and the remaining parent ion were exposed to D\(_2\)O for 2 minutes at 5\(\times\)10\(^{-7}\) mbar. The result is presented in figure 8.8. Using only the mono-isotopic parent ion for this purpose ensures that all isotopic peaks that are observed are due to H/D exchange and not due to the natural abundance of other isotopes (\(^{13}\)C, \(^{17}\)O, \(^{18}\)O and \(^{15}\)N). The fragment \([P_3D_4-H_2O]^{ox+}\) (1) resulting from the loss of H\(_2\)O does not exhibit H/D exchange behaviour, which proofs that this fragment does not contain a mobile proton. Scheme 7.1 was proposed to rationalise the formation of an oxazolonium ion. Many of the other fragments (2, 4 and 7) do show H/D exchange behaviour. Mechanisms 7.2 and 7.3 lead to oxazolonium fragment ions that should not exhibit H/D exchange behaviour because they do not contain mobile protons. However, many ions exhibit H/D exchange behaviour, as can be seen from the inserts in figure 8.8, and should therefore be protonated. Our findings indicate that mechanisms 7.2 and 7.3\(^9\) are not correct.

A nice feature of mechanisms 7.2 and 7.3 is that each cleavage of an ester or amide bond lead to only one charged fragment (oxazolonium ion).\(^9\) When introducing new mechanisms for the rearrangement of the ester and amide bonds that lead to protonated fragments, one should be aware that each rearrangement can lead to two charged fragments depending on the proton affinity of the fragments. This has as consequence that the interpretation of the MS/MS spectra becomes more complex.

If one assumes that only amide bonds are rearranged for which we propose scheme 8.1, the following protonated fragments will be obtained: \([PD-H_2O+H]^+\), \([P_2D_2-H_2O+H]^+\) (7), \([P_3D_2-H_2O+H]^+\) (2), \([D+H]^+\), \([PD_2+H]^+\) and \([P_2D_3+H]^+\) (4). These fragments have all been observed, except for \([PD-H_2O+H]^+\), \([D+H]^+\) and \([PD_2+H]^+\), and all exhibit H/D exchange activity. If an ester bonds is rearranged it is likely that this will lead to a fragment with an carboxylic acid endgroup and a fragment with an allylic endgroup. Such rearrangements have been observed.
This rearrangement of the ester bond results in protonated fragments $\text{[D-H}_2\text{O+H]}^+$, $\text{[PD}_2\text{-H}_2\text{O+H]}^+$, $\text{[P}_2\text{D}_3\text{-H}_2\text{O+H]}^+$, $\text{[PD+H]}^+$, $\text{[P}_2\text{D}_2\text{+H]}^+$ and $\text{[P}_3\text{D}_3\text{+H]}^+$. Fragments $\text{[PD+H]}^+$, $\text{[P}_2\text{D}_2\text{+H]}^+$ and $\text{[P}_3\text{D}_3\text{+H]}^+$ have not been observed. Moreover, fragments $\text{[PD}_2\text{-H}_2\text{O+H]}^+$ (9) and $\text{[P}_2\text{D}_3\text{-H}_2\text{O+H]}^+$ (5) are observed in the MS/MS spectrum but do not exhibit H/D exchange activity. It is therefore more likely that fragments $\text{[PD}_2\text{-H}_2\text{O+H]}^+$ (9) and $\text{[P}_2\text{D}_3\text{-H}_2\text{O+H]}^+$ (5) are the result of an amide rearrangement (scheme 8.1) followed by the loss of $\text{H}_2\text{O}$ from one of the endgroups to an oxazolonium ion in scheme 7.1. These results indicate that only amide bonds are rearranged upon CAD of these polymers and not ester bonds.

Figure 8.8  MS/MS of mono-isotopic parent ion $\text{[P}_3\text{D}_4\text{+H]}^+$ with $E_{\text{kin.lab}}=180$ eV followed by 2 minutes H/D exchange at $5\cdot10^{-7}$ mbar. The numbered fragments are $\text{[P}_3\text{D}_4\text{-H}_2\text{O]}^{\text{ox}+}$ (1), $\text{[P}_3\text{D}_3\text{-H}_2\text{O+H]}^+$ (2), $\text{[P}_3\text{D}_3\text{-2H}_2\text{O]}^{\text{ox}+}$ (3), $\text{[P}_2\text{D}_3\text{+H]}^+$ (4), $\text{[P}_2\text{D}_3\text{-H}_2\text{O]}^{\text{ox}+}$ (5), $\text{[P}_2\text{D}_3\text{-2H}_2\text{O]}^{\text{ox}+}$ (6), $\text{[P}_2\text{D}_2\text{-H}_2\text{O+H]}^+$ (7), $\text{[P}_2\text{D}_2\text{-2H}_2\text{O]}^{\text{ox}+}$ (8), $\text{[PD}_2\text{-H}_2\text{O]}^{\text{ox}+}$ (9) and $\text{[PD}_2\text{-2H}_2\text{O]}^{\text{ox}+}$ (10).
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Scheme 8.1 Newly proposed rearrangement of the amide bond leading to protonated fragments.

8.3.4. Other hyperbranched polyesteramides

H/D exchange experiments have also been performed with hyperbranched polyesteramide polymers based on 1,2-cyclohexane dicarboxylic acid anhydride (see figure 7.1 for the structure). The polymer shows similar DoDIP and H/D exchange behaviour as the phthalic acid containing polymer described in the previous sections indicating that this polymer also contains alternating and non-alternating oligomers. Schemes 7.1 and 8.1 are therefore also valid for the hyperbranched polyesteramide based on 1,2-cyclohexane dicarboxylic acid anhydride. The oxazolonium ions of the 1,2-cyclohexane dicarboxylic acid based polymers can have a protonated structure as shown in scheme 7.4. However, the H/D exchange experiments and the MS$^3$ experiments described in chapter 7 demonstrate that these ions are not formed.

Hyperbranched polymers based on succinic acid and glutaric acid anhydride (see figure 7.1 for their structure) do not exhibit H/D exchange activity. Additionally, their MS$^2$ behaviour shows similarities with the MS$^2$ behaviour of the non-alternating oligomers for which an example of the phthalic acid containing polymer is shown in figure 8.5. Fragments due to the loss of one D are very low in intensity. The fragment that is structurally similar as fragment [P$_2$D$_5$+H]$^+$ is not observed. This indicates that the hyperbranched polyesteramides based on succinic acid and glutaric acid contain a much higher fraction of non-alternating oligomers (i.e. contain many amine bonds with high proton affinity). DoDIP experiments have not been performed.

The results described above indicate that the synthesis of hyperbranched polyesteramides results in polymers containing an isomeric composition that depends on the monomers used in the polymerisation reaction. Two types of polymers are being formed of which one contains a mixture of oligomers with an
alternating and non-alternating monomer sequence (phthalic acid and 1,2-cyclohexane dicarboxylic acid anhydride). The other contains mainly oligomers with a non-alternating monomer sequence (succinic acid and glutaric acid anhydride).

8.4. Conclusions

A novel MS^n approach is used to determine whether oligomers from the hyperbranched polyesteramides based on phthalic acid anhydride and 1,2-cyclohexane dicarboxylic acid anhydride studied in this work consist of different isomeric structures. The method is called DoDIP: Dissociation of Depleted Ion Populations. The experiment starts with MS/MS to increase the relative abundance of isomeric structure that fragments at relatively high appearance energy followed by a second MS^2 experiment on the same parent ion. This approach was used to demonstrate that oligomers that were expected to have an alternating sequence consist of a mixture of isomers with an alternating and non-alternating monomer sequence. The method requires that the isomeric structures have a difference in the appearance energy. The same conclusion was drawn from another experimental approach that uses gas phase H/D exchange experiments. Gas phase H/D exchange experiments lead to a bimodal isotopic distribution of \([P_nD_{n+1}+H]^+\) and \([C_nD_{n+1}+H]^+\) indicating the presence of at least two isomeric structures. One of the isomeric structures does exhibit H/D exchange behaviour and the other does not. The non-exchanging isomer is a result of the non-alternating sequence of the oligomer that contains a tertiary amine with a relatively high proton affinity. Oligomers with alternating sequences only consist of amide and ester bonds with a relatively low proton affinity. Functional groups with high proton affinity reduce the mobility of protons making H/D exchange less facile. The structures of the alternating and non-alternating oligomers were confirmed with additional MS^2 experiments after separation with gas phase H/D exchange.

Hyperbranched polyesteramides based on succinic acid and glutaric acid did not exhibit H/D exchange behaviour. Their MS/MS behaviour is very similar to oligomers with a non-alternating monomer sequence, which indicates that these polymers contain a high fraction of non-alternating oligomers.

Mechanisms 7.2 and 7.3 that were proposed for the cleavage of the ester and amide bond need to be revised. It is concluded that two types of fragments are being formed upon CAD based on gas phase H/D exchange experiments on the fragments that are obtained after MS^2. One type of ions is protonated and is proposed to result from a rearrangement of the amide bond. The other fragments
are due to amide bond rearrangements with an additional loss of H$_2$O from the endgroups, which leads to an oxazolonium ion.

The oligomer series $[P_nD_{n+1}+H]^+$ and $[C_nD_{n+1}+H]^+$ consist of alternating and non-alternating oligomers. Consequently, the non-alternating oligomers must contain OH and COOH endgroup functionalities. The presence of carboxylic acid endgroups will have an influence on, for example, cross-linking when used as cross-linkers for powder coatings.