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APPLICATION NOTE

MALDI In-Source Decay Mass Spectrometry of Polyamidoamine Dendrimers

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Abstract

We report using MALDI-ISD (in-source decay) mass spectrometry (MS) to characterize highly branched synthetic polymers of polyamidoamine (PAMAM) dendrimer. This inherently monodisperse polymer possesses dendritic branches networked by tertiary amines and an amide functionality in each repeating unit. Among various ISD matrices examined, 2,5-DHB was the most efficient, yielding 33 fragments produced by single- or multiple-bond cleavages. Detailed analysis revealed that cleavages at tertiary amine sites (*S*- and *E*-type fragments) were the most pronounced, with various other cleavages around amide groups. The fragmentation mechanism appeared to follow the radical-induced dissociation pathway. In addition, the matrix dependence of PAMAM MALDI-ISD differed from that of peptides/proteins. The observed fragments provided rich structural information, which was suitable to characterize dendritic polymers.

Key words: MALDI, In-source decay (ISD), Radical-induced dissociation, PAMAM dendrimers, Branched synthetic polymers

Introduction

In recent years, the MALDI-ISD (in-source decay) method has been extensively investigated for characterizing biopolymers, such as peptides, proteins, oligosaccharides, carbohydrates, oligonucleotides, and DNAs [1–5]. In analyzing peptides/proteins, MALDI-ISD mainly forms ***c***- and ($z+2$)-type ions, suggesting that a radical-induced pathway is deeply involved in the fragmentation mechanism [6, 7], as found in electron capture dissociation (ECD) [8–10]. Previous studies revealed that intermolecular hydrogen transfer from matrix molecules to analytes and MALDI plume dynamics play important roles in the ISD of peptides/proteins [3, 7]. Thus, the ISD process appears to be matrix-dependent, so the choice of matrix determines the fragmentation efficiency and pattern [5, 11–14]. On the other hand, MALDI-ISD has features differentiated from the common PSD (post-source decay) process that occurs via a thermally-activated pathway [15]. In PSD, dissociation of large analytes, (i.e., molecules with many vibrational degrees of freedom) is limited because of rapid intramolecular vibrational energy redistribution (IVR) [16].

Another field that anticipates successfully applying MALDI-ISD MS is characterizing synthetic polymers; however, few cases are known to date [17]. One limit to broadly applying MALDI-ISD to synthetic polymers is the polydispersed nature of synthetic polymers. MALDI-ISD MS generally requires relatively pure samples because of the lack of a precursor isolation procedure.

In the present study, we examined MALDI-ISD MS to characterize polyamidoamine (PAMAM) dendritic polymers. PAMAM dendrimers are a class of synthetic polymers with a dendritic architecture consisting of an alkyl-diamine core and tertiary amine branches with a repeating amide functionality. In addition, PAMAM dendrimers inherently possess a high degree of monodispersity [18–21]. The dendritic structure and various surface functionalities of PAMAM dendrimers have many important applications in

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materials science and biotechnology, including drug delivery and biomedical imaging. In parallel with their growing applications, the demand for a suitable characterization method is also increasing. Consequently, many tandem MS techniques such as collision-induced dissociation (CID), ECD, and electron detachment dissociation (EDD) have been successfully examined for analyzing PAMAM dendrimer structure [19–21]. In this application note, we demonstrate that MALDI-ISD MS provides a simple and inexpensive means to characterize dendritic polymers, yielding rich structural information without tandem MS techniques.

Experimental

Third generation PAMAM-amidoethanol dendrimers with a 1,12-diaminododecane core (see Scheme 1, monoisotopic mass=7076.5 Da), and third generation poly(propylene imine) dendrimers with a 1,4-diaminobutane core (see Scheme S1 in Supplemental Information) were purchased from Sigma-Aldrich (Suwon, Korea). Matrices, including 2,5-dihydroxybenzoic acid (2,5-DHB), α-cyano-4-hydroxycinnamic acid (CHCA), 1,5-diaminonaphthalene (1,5-DAN), sinapic acid (SA), 5-amino salicylic acid (5-ASA), 5-formyl salicylic acid (5-FSA), and 5-nitrosalicylic acid (5-NSA), were also purchased from Sigma-Aldrich.

To prepare samples, 10 wt% PAMAM dendrimers in methanol were dissolved in deionized water to make a 10 μmol/L solution. A matrix solution was prepared in water/acetonitrile (1:1 vol/vol) with 0.1 % trifluoroacetic acid at 10 mg/mL. Then, 5 μL of the dendrimer solution was mixed with 5 μL of the matrix solution. A 1 μL drop of the mixed solution was deposited onto a stainless-steel MALDI plate and left to dry in air at room temperature.

MALDI-ISD mass spectra were taken using a MALDI-TOF mass spectrometer equipped with a 355 nm laser (Autoflex Speed series; BrukerDaltonics, Leipzig, Germany). Mass spectra were acquired in positive reflectron mode. The UV laser fluence was typically 15 % above the ionization threshold fluence.

Results and Discussion

MALDI-ISD of PAMAM Dendrimers with Various Matrices

To assign MALDI-ISD spectra, we employed the fragment notations that we previously proposed for PAMAM dendrimers (see Scheme 1c) [19–21]. In brief, we used the form $G_n(l)$, where $_n$ is the generation number of the cleavage site and l is the fragmentation type (***a***/***x***, ***b***/***y***, ***c***/***z***, $\boldsymbol{S}_{in/out}$, or $\boldsymbol{E}_{in/out}$). The notations of ***a***/***x***, ***b***/***y***, or ***c***/***z*** are given in the same manner as those used for peptide fragmentation since the PAMAM dendrimer contains an amide functionality in a repeating unit as in peptides [19]. $\boldsymbol{S}_{in\ or\ out}$ and $\boldsymbol{E}_{in\ or\ out}$ denote cleavages at tertiary amine sites, where ***S*** and ***E*** stand for the start and end, respectively, of each generation repeat unit and $_{in}$ or $_{out}$ indicates whether the fragment includes the dendrimer core.

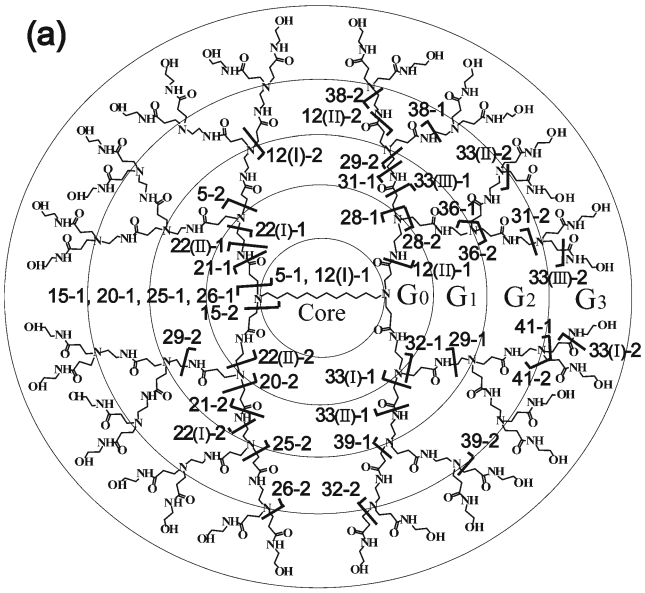
Figure 1a shows the ISD spectrum of third generation PAMAM dendrimers obtained using 2,5-DHB matrix. Detailed analysis revealed that ISD created a large number of fragment peaks (33 peaks), which appeared along with singly protonated dendrimers at *m/z*=7077.5 (43 in Figure 1) in the mass spectrum. The assigned peaks are listed in Supplemental Table S1.

The fragments can be classified into three groups based on the number of cleavages necessary to generate each fragment peak (Groups A, B, and C). The peaks observed at *m/z* 5358.8 ($[G_0(S_{in})+2H]^+$, **27**), 6275.2 ($[G_1(S_{in})+2H]^+$, **34**), 6329.4 ($G_1(b)^+$, **35**), 6733.5 ($[G_2(S_{in})+2H]^+$, **40**), and 6962.8 ($[G_3(S_{in})+2H]^+$, **42**) were assigned to single-bond cleavages (Group A, see the inset of Figure 1a). The dominant cleavages occurred at tertiary amine sites, forming $G_{0\text{-}3}(S_{in})$. The Group A peaks, *S*-type fragments, are particularly important in providing structural information of the dendrimer. For example, differences in *m/z* between the peaks **27**, **34**, **40**, **42**, and **43** ($[M+H]^+$) can be utilized for identification of surface functional group, monomer unit, and dendrimer branch size. An amide bond (–C(O)–NH–) cleavage was also observed as a minor channel; $G_1(b)^+$ at *m/z* 6329.4 (**35**).

The second group of fragments (Group B) was generated by cleavage of two bonds (17 peaks). The annotations for Group B fragments are given in Scheme 1a. Some peaks could be assigned in two or three different ways, for example, **22(I)**, **22(II)**, and **33(I)**, **33(II)**, **33(III)**. Most fragmentations in Group B included at least 1 cleavage at tertiary amine. In addition, a variety of ***a***/***x***, ***b***/***y***, and ***c***/***z***-type cleavages yielded $G_{0\text{-}3}$(***x***, ***y***, or ***z***) fragments, e.g., $[M-G_0(\boldsymbol{y})-G_1(\boldsymbol{y})+H]^+$ (**21**) and $[M-G_0(E_{out})-G_1(\boldsymbol{z})-H]^+$ [**22(I)**]. The third group fragments (Group C) was generated by cleavage of three bonds (11 fragment peaks, Scheme 1b). The relative extent of multiple cleavages was influenced by the MALDI laser fluence.

As ISD performance is generally matrix-dependent, we examined MALDI-ISD mass spectra using other matrices. Figure 1b and c display the ISD mass spectra obtained with CHCA and 1,5-DAN, respectively. The mass spectra obtained with SA, 5-ASA, 5-FSA, and 5-NSA are presented in Supplemental Figure S1. As shown in Figure 1b (see also Supplemental Table S1), MALDI-ISD with CHCA yielded 24 fragment peaks: 4 peaks in Group A, 12 peaks in Group B, and 8 peaks in Group C. Although fragmentation was not as pronounced as 2,5-DHB, the CHCA ISD mass spectrum yielded structural information comparable to 2,5-DHB ISD. The performances of the other matrices, however, were not satisfactory for characterization. For example, 1,5-DAN yielded several fragments in the high *m/z* region along with a cluster of peaks between *m/z* 1000 and 1500, which were not informative. In addition, the fragmentation efficiencies of SA, 5-ASA, 5-FSA, and 5-NSA matrices were low, not useful for characterizing PAMAM dendrimers.

In previous studies, ISD of peptides/proteins has been proposed to proceed mainly through hydrogen transfer from



◀ **Scheme 1.** **(a)** Structure of the third generation PAMAM-amidoethanol dendrimer with a 1,12 diaminododecane core, and cleavage sites corresponding to Group B (2,5-DHB) are all denoted; **(b)** cleavage sites corresponding to Group C (2,5-DHB) are denoted; **(c)** the previously proposed fragmentation notations for PAMAM dendrimers [19]. Two cleavage pairs and three cleavage triplets are labeled as X-1/X-2 or Y-1/Y-2/Y-3, respectively, e.g., 5-1/5-2 in **(a)**

matrix to analyte, producing ***c***- and ***z***-type ions via radical-induced pathways [7, 11–13]. The thermochemical properties of matrices corroborated with the resulting ISD efficiencies, following a well-established rank for hydrogen-donating abilities: 1,5-DAN>5-ASA>2,5-DHB>SA>CHCA [11, 12]. Our results for PAMAM dendrimers, however, do not follow this order. The hydrogen-accepting ability of matrix molecules, such as 5-FSA and 5-NSA, also influences ISD of peptides, forming ***a***- and ***x***-type fragment ions via hydrogen abstraction [14]. When these matrices (5-FSA and 5-NSA) were employed, however, PAMAM dendrimers underwent ISD only with poor fragmentation efficiency.

Fragmentation Characteristics of PAMAM Dendrimer ISD

The total abundance of each ISD fragment-type is analyzed for mass spectra produced with 2,5-DHB, CHCA, and 1,5-DAN matrices. For all the matrices, *S*/*E*-type fragmentations at tertiary amine sites prevailed, accounting for more than 80 % of all fragmentations. Formation of abundant *S*- and *E*-fragments agrees with previous ECD results [19, 20], suggesting that *S*/*E*-type cleavages are products of a radical-induced pathway occurring via homolytic bond cleavage. Radical-induced homolytic bond cleavages were also observed previously in ECD and activated electron photo-detachment experiments for other synthetic polymers [22, 23]. The relative abundances of ***a***/***x***, ***b***/***y***, and ***c***/***z***-type ISD fragments, however, were lower than in ECD; less than 10 % of total *S*/*E* fragment abundance, but it was 60 %–85 % in the previous ECD experiment [19]. The observed ***a***/***x*** and ***c***/***z***-type ISD fragments are very likely to be formed through hydrogen transfer from matrix to analyte via radical-induced pathways [7, 11–13].

In a comparative ISD study of third generation poly (propylene imine) dendrimers with a 1,4-diaminobutane core (Supplemental Scheme S1) using a 2,5-DHB matrix, extensive cleavage was also observed at tertiary amines. As this dendrimer contains only tertiary amines as a reactive site, it can be deduced that the backbone itself, a tertiary amine network, is intrinsically susceptible to MALDI-ISD cleavage, most likely through a radical-induced pathway. The radical-induced dissociation at tertiary amine sites in the PAMAM dendrimer is also supported by previous neutralization-reionization studies, wherein hypervalent nitrogen radicals, e.g., $(CH_3)_2NH_2$•, formed by collisional reduction readily dissociated via N−C bond cleavages [24]. A further attempt to obtain PSD spectra of third generation PAMAM

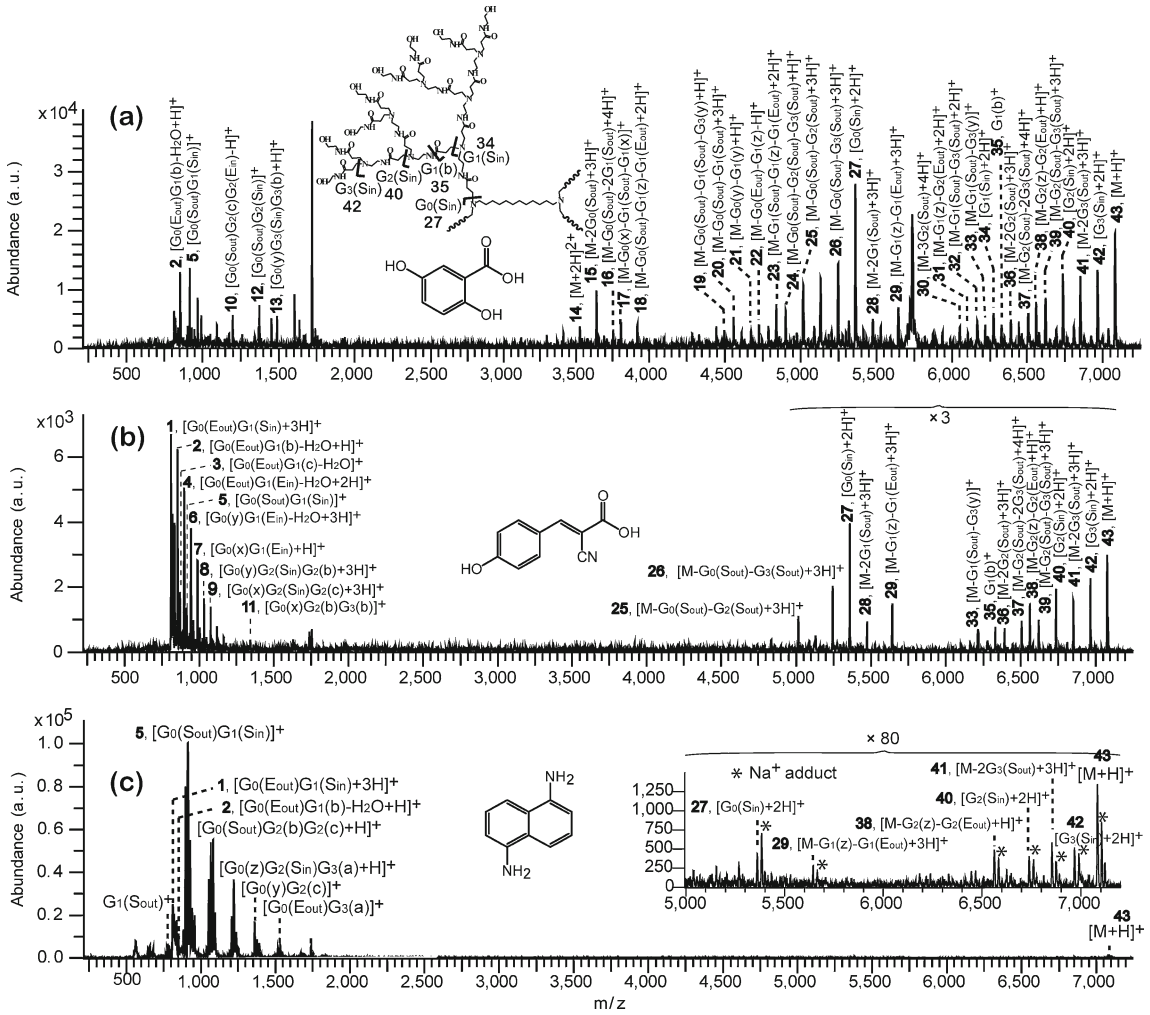


Figure 1. MALDI-ISD mass spectra of third generation PAMAM dendrimers obtained with **(a)** 2,5-DHB, **(b)** CHCA, and **(c)** 1,5-DAN matrices. In the inset of **(a)**, the fragmentation locations with a single-bond cleavage are denoted. The numbers in bold refer to the peaks in Supplemental Table 1. The ISD spectra given here were obtained by accumulating over 1000 laser shots

dendrimers using 2,5-DHB and CHCA matrices in LIFT-TOF/TOF mode was not successful at all [25]. This suggests that thermal activation induced in the MALDI process is not sufficient to fragment the tertiary amine backbone of this large dendrimer (7076.5 Da) due to rapid IVR. Considering these observations, radical-induced dissociation promoted by matrix molecules appears to be the major pathway for ISD of PAMAM dendrimers.

As described above, the overall ISD efficiency of PAMAM dendrimers did not follow the order of hydrogen-donating or -accepting abilities of the matrices, in contrast to previous peptide/protein ISD studies [7, 11–13]. In view of the intermolecular hydrogen transfer mechanism, the observed discrepancy may be explained as follows. The highly branched structure may hinder matrix molecules from accessing interior amide groups, limiting the formation of intermolecular hydrogen bonds in matrix crystals. A molecular dynamics study of PAMAM dendrimers with amine terminals (generations 1 through 11) showed that the outer sub-generations penetrated substantially into the interior of the dendrimer molecules [18]. The penetrating branches may form hydrogen bonds with interior amide groups, and thus the chances of matrix molecules locating nearby amide groups for hydrogen transfer becomes substantially low. This may account for the lower ISD abundance of amide bond cleavages than in ECD.

Conclusions

We used MALDI-ISD to analyze the structure of third generation PAMAM dendrimers that are highly-monodisperse inherently. The ISD of the dendrimer, in particular when using a 2,5-DHB or CHCA matrix, produced extensive and various fragment ions, mainly created by radical-induced pathways. The ISD results demonstrated that MALDI-ISD MS displays rich structural information about PAMAM dendrimers in a single mass spectrum, making it a versatile analytical tool for characterizing synthetic polymers with a highly branched dendritic architecture. MALDI-ISD is likely also applicable to characterizing other PAMAMs with different generation numbers and surface functional groups.

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