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ARTICLE TYPE

Poly-Amidoamine Structures Characterization: Amide Resonance Structure Imidic Acid (HO-C=N) and Tertiary Ammonium

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The fluorescence emission phenomena of ploy-amidoamine (PAMAM) have been found and determined in recent decades. There were amide, primary amine, and tertiary amine in the PAMAM, which were not the traditional typical fluorescence emission groups. The fluorescence emission groups or mechanism of PAMAM were cared. This paper tested the NMR (15N NMR, 13C NMR, 1H NMR, and N-H 2D NMR), IR, and MS of PAMAM. The tested results proved there were amide resonance structure imidic acid and tertiary ammonium exits in PAMAM. The imidic acid and tertiary ammonium was the new groups found in PAMAM, which might help to explain the intrinsic-fluorescent phenomena.

Introduction

Poly-amidoamine (PAMAM) dendrimers were firstly synthesized by Tomalia in 1985.[1] PAMAM dendrimers have water dissolvable and inside holes [2], several modifiable chemical groups [3], well biocompatible [4], nano particles packaged able [5], and small molecules or drugs delivery abilities [6]. In recent years, the fluorescence emission phenomena of PAMAM have been observed [7], which caused the interests and cares. The PAMAM dendrimers have amide, primary amine, and tertiary amine. There were no traditional typical fluorescence emission groups in PAMAM. And some other dendrimers such as Poly(amino ester)s[8b], Poly(propyl ether imine) [8e], poly(propyleneimine) (PPI) dendrimer, and poly(ethyleneimine) (PEI) dendrimer [9] can gave fluorescence emission, which were called intrinsic-fluorescent phenomena.[10] So the fluorescence emission center and mechanism of PAMAM have been notified and studied by many research groups [8].

In this paper, the PAMAM produced amide resonance structure imidic acid (HO-C=N) under some situations, which may connected with the fluorescence emission of PAMAM. The 15N NMR, 1H NMR, 13C NMR, N-H 2D NMR, and IR spectra have determined this amide resonance structure imidic acid and the tertiary ammonium exit in the structure of PAMAM, which new proved groups found in PAMAM.

The amide resonance structure imidic acid (HO-C=N) have been studied by experiments [9] and quantum chemical study[10]. The amide/ imidic-acid were a kind of tautomers. The tautomars ratios[11] depend on temperature, solvent, and pH. The amide/imidic-acid transition in PAMAM might connect with these elements. The amide/imidic-acid mechanism can explain some of the influence on amide/imidic-acid transition, such as pH values. The amide resonance structure imidic acid exits in PAMAM derivatives that concerned with fluorescence emission have related report.[12] The imidic acid and tertiary ammonium structure were new found in PAMAM, which might help for explain the intrinsic-fluorescent phenomena.

Results and Discussions

PAMAM (Figure 1) gave weak fluorescence when synthesized initially. But the PAMAM dendrimers staled in air of added acid, and then PAMAM can give fluorescence emission. The fluorescence emission phenomena of PAMAM have been proved by many research groups.[7-10]

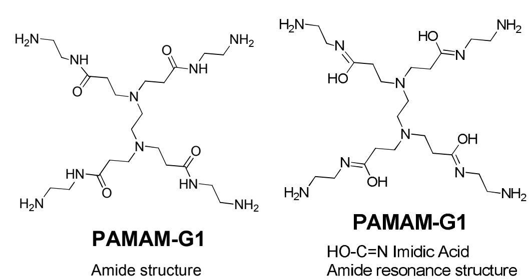

Fig. 1 The PAMAM-G1 amide structure and PAMAM-G1 amide resonance structure imidic acid.

In order to determine the chemical structure of PAMAM fluorescence emission centers, NMR (15N NMR; 1H NMR; 13C NMR; N-H 2D NMR), MS, and IR spectra were tested to characterize the structures of PAMAM-G1.

The PAMAM dendrimers were sticky liquid at room temperature. The pure PAMAM-G1 gave strong fluorescence emission, but the PAMAM solution gave weak fluorescence. The traditional NMR was carried in solution, which might influence the fluorescence emission center of PAMAM. So the pure PAMAM-G1 was used to carry out NMR to give the pure states PAMAM-G1 structure information. 15N NMR; 1H NMR; 13C NMR; N-H 2D NMR; and IR of pure PAMAM-G1 were tested.

The 15N NMR was carried by 600MHz NMR liquid spectrometer. The pure PAMAM-G1 was sticky liquid, and sample temperature tuned at 60℃ in the 15N NMR testing. The nature 15N abundance was 0.36 % [13], so the 15N NMR was took by pure liquid PAMAM-G1 sample without adding solvents. The 15N NMR testing time was about 30 hours. There were three kinds of N atoms in the PAMAM-G1, which were amide, primary amine, and tertiary amine.

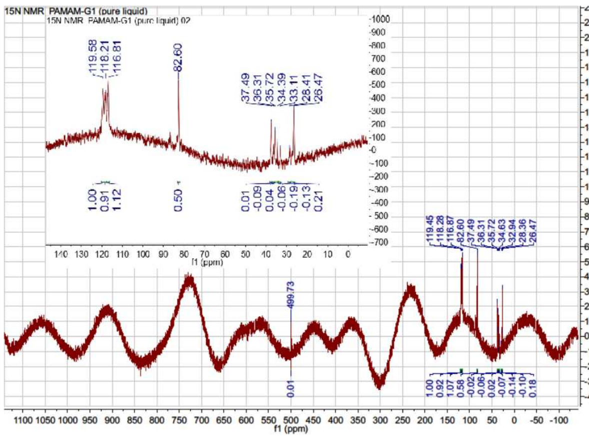

Fig. 2 The 15N NMR spectra of PAMAM-G1 at pure liquid state . δ (600MHz 15N NMR): 119.58, 118.21, 116.81 (amide); 82.60 (tertiary amine); 26.47 to 37.49 (amine and ammoniums).

The Figure 2 gave the 15N NMR spectra of pure PAMAM-G1. The 15N NMR was tested range from -100 to 1000 ppm. The peaks at about 82.60 were attributed to N atom of tertiary amine. The peaks ranges from 26.47 to 37.49 were attributed to primary amine and tertiary amine. Peaks range from 34.39 to 37.49 were attributed to ammonium (primary ammonium and tertiary ammonium), which show there exit different levels protonation of N atoms in primary amine or tertiary amine.

The three peaks 119.58, 118.21, and 116.81 were attributed to N atoms of amide. The integrate areas ratios were 1.00/0.91/1.12. The amide N atoms NMR peaks were split into three peaks, which show there were three states of amide N atoms. The three peaks correspondent to the resonance structures of amide in PAMAM-G1, and one of these states was imidic acid. At range 200 to 1000 ppm, there were 15N NMR 499.73 small peaks and this peak was attributed to N-O bond, which was very weak and might imidic acid hydroxyl connected with amine ion to form salts. The N-O bond peak was so weak that excluded the possibility of PAMAM have formed lots of oxime, which excluded the reverse reaction of oxime/amide Beckmann rearrangement.

N-H 2D NMR (Figure 3) show the (1H/15N NMR) 7.59, 119.72 peak. This peak was correspondent to the N-H 2D-NMR of amide. There were three peaks 116.81, 118.21, 119.58 in 15N NMR spectrum. These show the peak 119.72 (in N-H 2D NMR) or peak same as 119.58 (in 1D N NMR) connected with one H atom; the other nearby amide peaks 116.81 and 118.21 (in 1D N NMR) were not connected with H atom, which prove the amide exit the states that N atom has no bond with H atom. The amide resonance structure imidic acid has N atom without bond with H atom. So the 116.81 and 118.21 peaks were attributed to imidic acid state and middle transitional state of amide.

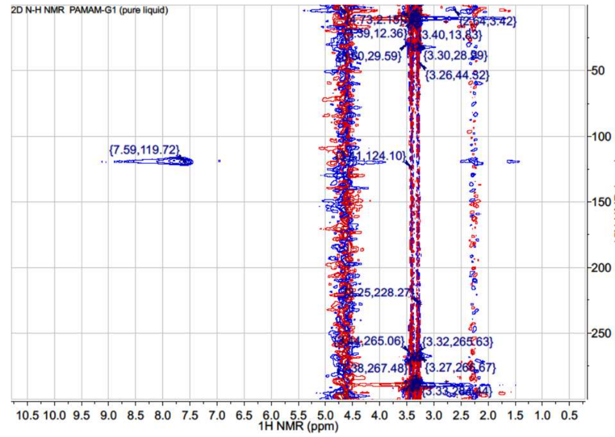

Fig. 3 The 15N-1H 2D (two dimensions) NMR of PAMAM-G1 at pure liquid state. δ (600MHz 2D 1H/15N NMR): 7.59/119.72 (amide)

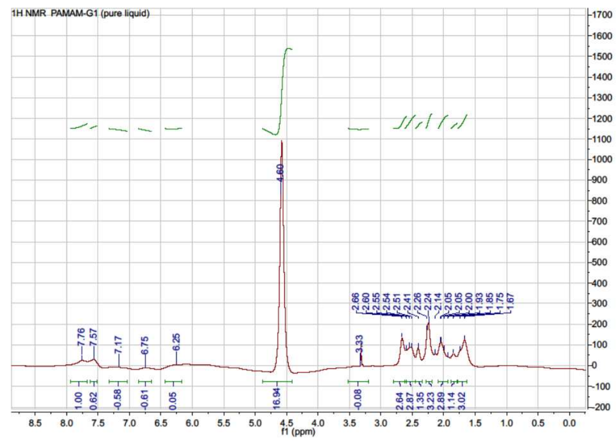

Fig. 4 The 1H NMR spectrum of PAMAM-G1 at pure liquid state. δ (600MHz 1H NMR): 7.76-7.57 (amide);7.17-6.25(ammoniums); 4.60 (hydroxyl of HO-C=N) ; 1.67-2.66 (amine 2H-N)

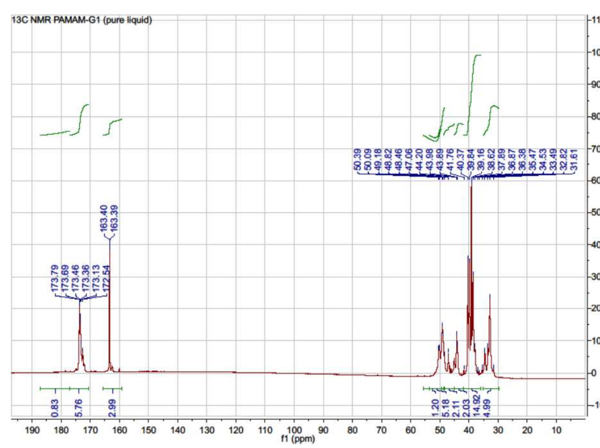

Fig. 5 The 13C NMR spectrum of PAMAM-G1 at pure liquid state. δ (600MHz 13C NMR): 172.54-173.79 (amide); 163.39-163.40 (imidic acid HO-C=N); 31.61-50.39 (methylene)

1H NMR (Figure 4) has strong peak at 4.60, which was attributed to hydroxyl NMR peaks. The pure PAMAM-G1 tested without solvent added, which exclude H_2O or other hydroxyl. The H NMR show there was HO hydroxyl structure formed in the PAMAM. This show the oxygen connected with H atoms. The

Hydroxyl was the HO- group of amide resonance structure imidic acid (HO-C=N).The peaks at 7.17; 6.75; 6.25 were attributed to ammonium H atom, which were primary ammoniums and tertiary ammoniums. The peak 7.57 was attributed to amide.

13C NMR (Figure 5) have 163.39-163.40 and 172.54-173.79 peaks to prove there were exits two states of amide C atoms. The two states were amide and imidic acid.

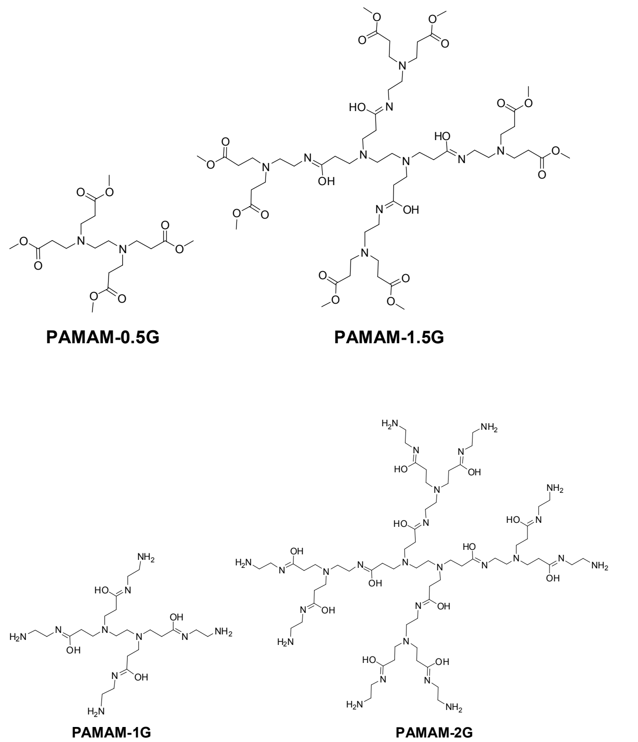

Fig. 6 The structure (amide resonance structure imidic acid formula) of PAMAM-0.5G, PAMAM-1.5G, PAMAM-1.0G, and PAMAM-2.0G.

PAMAM-0.5G, PAMAM-1G, PAMAM-1.5G, and PAMSM-2.0G (Figure 6) IR spectra (Figure 7) of gave the carbonyl and double bonds information. There exit 1733 peak in IR of PAMAM-0.5G, which were attributed to carbonyl of ester. There exit 1733 and 1659 peaks in IR of PAMAM-1.5G. The 1733 were attributed to carbonyl of ester. The 1659 were attributed to double bonds. There exit 1647 and 1644 peaks in IR of PAMAM-1.0G and PAMAM-2.0G respectively. The 1647 and 1644 peaks were attributed to double bonds. But there were no 1733 peaks in the IR of PAMAM-1.0 G and PAMAM-2.0 G, which show there were no carbonyl exits in PAMAM-1.0 G and PAMAM-2.0 G. The 1644-1659 double bonds IR peaks should be the C=N of imidic acid of PAMAM.

MS spectra (Figure 8) of PAMAM-G1 gave $[M+2H]^{2+}$ peaks. There were amide resonance structures and amine protonation changed in PAMAM-G1, but no other chemical groups produced in PAMAM-G1. The amide resonance structure imidic acid not changes the molecular weight of PAMAM. The MS spectra cannot exclude the possibility of amide resonance structure imidic acid. The molecules from 517.4 to 520.3 show the protonation, which can determine that there ammoniums exited in PAMAM structure.

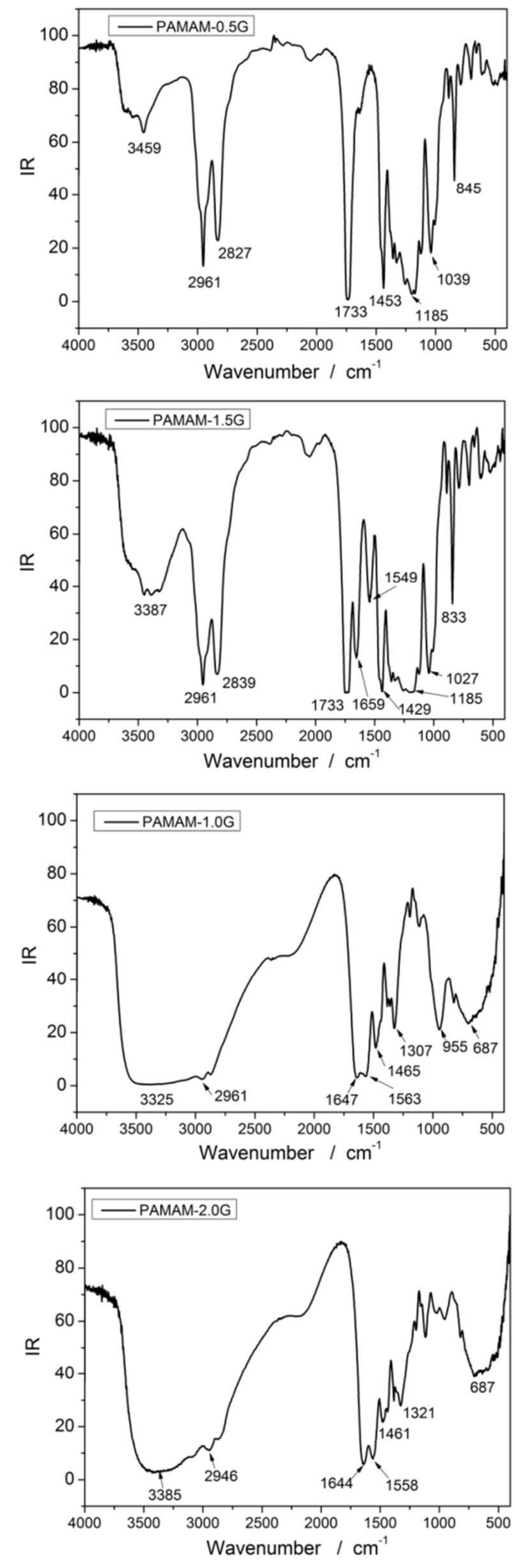

Fig. 7 The IR spectra of PAMAM-0.5G, PAMAM-1.5G, PAMAM-1.0G, and PAMAM-2.0G.

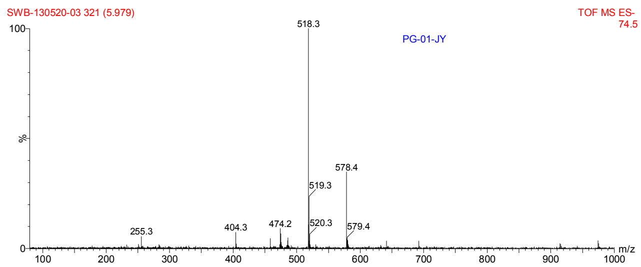

Fig. 8 The MS spectra (two samples) of PAMAM-G1 (Figure 1) (calculated: 516.4 [M], 517.4 $[M+H]^+$, 518.4 $[M+2H]^{2+}$; Found: 518.3 $[M+2H]^{2+}$, 519.3 $[M+3H]^{3+}$, and 520.3 $[M+4H]^{4+}$)

Table 1 The NMR and IR spectra data of pure PAMAM liquid sample.

Spectra [a]	Figs	Primary amine or ammonium	Tertiary amine or ammonium	Amide	Imidic acid (HO-C=N)	Ester (C=O)
15N NMR	Fig. 2	26.47-37.49	82.60	119.58	116.81; 118.21;	
1H NMR	Fig. 4	(1.67-2.66 amine) (7.17; 6.75; 6.25 ammonium)		7.57, 7.76	4.60	
1H/5N 2D NMR	Fig. 3			7.59/119.72		
13C NMR	Fig. 5	31.61-50.39	172.54-173.79	163.39-163.40		
IR PAMAM-0.5G	Fig. 7				-	1733
IR PAMAM-1.5G	Fig. 7	3387			1659	1733
IR PAMAM-1.0G	Fig. 7	3325			1647	
IR PAMAM-2.0G	Fig.7	3385			1644	

[a] Footnote : unit of NMR (ppm), IR (cm^{-1}).

These spectra data in Table 1 (15N NMR; 1H NMR; 13C NMR; N-H 2D NMR, IR, and MS) can determine that there amide resonance structure imidic acid exits in the PAMAM. This imidic acid structure (Figure 9) might be the fluorescence emission group, which was rigid co-plane structure, has C=N double bonds, p-π conjugated structure, and exits donor electronic group hydroxyl. The imine C=N double bonds can give fluorescence emission in some conditions.[14] The C=N in imidic acid was similar to C=N in imine, which can give fluorescence emission. There exit amide; imidic acid; primary amine; primary ammonium; tertiary amine [15]; and tertiary ammonium in PAMAM show in Figure 9. The characterized structures connected with fluorescence emission properties, which will help for explain intrinsic fluorescent phenomena.

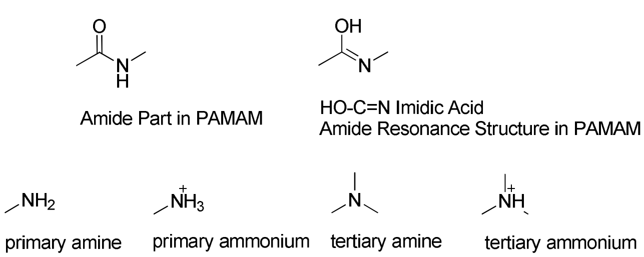

Fig. 9 The parts of amide (P-01); imidic acid parts (P-02); primary amine (P-03); primary ammonium (P-04); tertiary amine (P-05); and tertiary ammonium (P-06); in PAMAM..

Experimental Methods

1. Synthesis

PAMAM 0.5G, 1.0G, 1.5G, 2.0G, 3.0G, hyper-branch[1].

Methyl acrylate (MA) and ethylenediamine (EDA) were used as substrates.

(1) The Michael-addition of amine groups in EDA to MA under 50 ℃ in methanol solution (affords the dendritic product of 0.5 generation (G) with ester groups terminated).

(2) The amidation of the terminal ester groups of 0.5G dendrimer from dissolving in methanol solution by excessive EDA under 50 ℃ (affords the 1G dendrimer with terminal amine groups).

(3) Distillation of exceeded EDA under reduced pressure and washed by ethyl ether (gives the purified 1 G dendrimer). The PAMAM dendrimers are shown as yellow sticky liquid.

(4) Repeated step (1), (2), and (3) can get 1.5G, 2.0G, and 3.0G

(5) The equal moles MA and EDA were added into methanol. Keep stirring at 50 ℃ 24 h. Distillation of methanol. The hyper-branch PAMAM was got.

2. Instruments

NMR by BRUKER 600MHz NMR spectrometer. pure PAMAM-G1 sample (without adding solvents), keep 60℃ temperature, external standard method, testing time 30 h; IR by Nicolet 750 infrored spectrometer, pure PAMAM KBr smear; MS by LC-Q TOF MS spectrometer; Fluorescence by FluoMax-4 spectrofluorometer

Conclusions

The amide of PAMAM exits resonance structure imidic acid. The tertiary amine transferred to ammonium at some conditions. The 15N NMR, 13C NMR, 1H NMR, N-H 2D NMR, IR of pure liquid PAMAM-G1, and MS spectra proved imidic acid and tertiary ammonium structures in PAMAM. It can be concluded that there exit imidic acid structure and tertiary ammonium were new groups found in PAMAM, which may have relationship with the fluorescence emission of PAMAM. These results can help to explain and understand the intrinsic-fluorescent phenomena of dendrimers.

Notes and references

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