Photoluminescence from Amino-Containing Polymer in the Presence of CO₂: Carbamato Anion Formed as a Fluorophore

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Organic photoluminescent materials are important to many applications especially for diagnosis and detection, and most of organic photoluminescent materials contain fluorophores with extended conjugated structures. Recently some of amino-containing polymers without fluorophores with extended conjugated structure are observed to be photoluminescent, and one possible cause of the photoluminescence is oxidation of the amines. Here we show that photoluminescence can be produced by exposing a typical amino-containing polymer, polyethylenimine, to carbon dioxide. We demonstrate that carbamato anion formed via the reaction between the amine and carbon dioxide is a fluorophore; and the loosely-bound protonated water molecule can increase UV absorption but reduce the photoluminescence emission. Also carbamato anion shows solvent- and excitation wavelength-dependent emission of photoluminescence. The photoluminescence profile of carbamoto anion was discussed. These results will facilitate the understanding of photoluminescence observed from amino-containing materials and the design of new fluorophores.

Photoluminescent organic compounds such as proteins4–8 and dyes5–8 have been explored for bioimaging3, detection7,8, display5,6 and solar energy production11,12. Photoluminescence is a process of emission of photons occurred when fluorophores of certain compounds return from an excited state to a lower energy state, and most of the fluorophores usually have extended conjugated structures filled with delocalized electrons which absorb and emit photons, such as p-hydroxybenzylidene-imidazolidone units in fluorescent proteins, fluorescein, rhodamine, and vitamin B12,13,14. However, many types of amino-containing polymers, e.g., poly(amide amines)15–25, poly(amino esters)26,27, polyethylenimine (PEI)28,29, polyurea dendrimer30, without extended conjugated structures have been reported to be photoluminescent since amino-containing dendrimers were reported to be able to emit photoluminescence19,31. Some of these amino-containing polymers have been explored for bioimaging15,21,25,29,32.

Regarding the photoluminescence mechanisms of these amino-containing polymers, one explanation is that oxidation of amines by oxygen in air contributes to the photoluminescence16,20. Considering the feasible reactions between amines and CO₂33–36, it is natural to inquire whether the reaction between the amines and CO₂ from air contributes to photoluminescence observed from amine-containing polymers. Therefore we were motivated to investigate the photoluminescence behavior of amino-containing polymers in the presence of CO₂. Here we show that introduction of CO₂ into a solution of a typical amino-containing polymer, polyethylenimine (PEI) as described in Figure 1, can produce photoluminescence and demonstrate that carbamato anion formed via the reaction between the amine and carbon dioxide is a fluorophore; and the loosely-bound protonated water molecule can increase UV absorption but reduce the photoluminescence emission. Also carbamato anion shows solvent- and excitation wavelength-dependent emission of photoluminescence. The photoluminescence profile of carbamoto anion was discussed. These results will facilitate the understanding of photoluminescence observed from amino-containing materials and the design of new fluorophores.

Results

PEI, one of the polymers with the highest amine content, was chosen as a typical amino-containing polymer for the investigation. A suitable UV absorption spectrum could be observed form aqueous solution of PEI containing 50 mM ethylenimine (EI) unit after CO₂ bubbling. UV-Vis absorption and photoluminescence of the solution were measured after bubbling CO₂ at a flow rate of ~ 3 ml/min for 3 h followed by being kept under ambient condition for a certain time. Figure 2A(a) shows the typical UV absorption spectrum and photoluminescence

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spectrum of the aqueous solution of PEI being kept for 5 days after CO2 bubbling. Obviously a strong UV absorption at 364 nm and a strong photoluminescence emission at 470 nm with an excitation of 364 nm can be observed. In contrast, no UV absorption and relatively very weak photoluminescence with an excitation at 364 nm can be observed from the aqueous solution of PEI prepared freshly or being kept for 5 days without CO2 bubbling as shown in Figures 2A(b) and 2A(c). Also no UV absorption and photoluminescence could be
observed from water solution bubbled with CO₂, and an aqueous solution of PEI containing 50 mM EI unit after bubbling with N₂ to removing O₂ or at pH 7.0 close to pH value after CO₂ bubbling. Therefore the strong UV absorption and photoluminescence are caused by introducing CO₂ into the aqueous solution of PEI.

As described in Figure 1, the reaction between amine and CO₂ forms carbamato anion via intermediates possibly composed of the loosely-bound encounter complex and zwitterions. Here ¹³C NMR was applied to monitor the formation of carbamate in a solution of PEI in D₂O bubbled with CO₂. Figure 2B(b) is ¹³C NMR spectrum of a solution of PEI in D₂O containing 50 mM EI unit being kept for 5 days after CO₂ bubbling. The peak at 125 ppm is attributed to the dissolved CO₂, the only peak observed in ¹³C NMR spectrum of D₂O bubbled with CO₂ as shown in Figure 2B(a). The peak attributed to the carbamato anion appears at 160 ppm, and the molar ratio of carbamato anion to the EI unit is determined to be ca. 7.1 : 100 (see supplementary information). In a contrast experiment, Ba(OH)₂ was added to check whether HCO₃⁻ was formed based on that BaCO₃ is insoluble and barium carbamate is soluble. The addition of Ba(OH)₂ showed almost no effect on ¹³C NMR spectrum, so the amount of HCO₃⁻ formed is negligible. This is reasonable because the kinetic reaction constant of carbamate formation is five orders of magnitude higher than that of HCO₃⁻ formation. There are three types of amine in PEI, i.e., primary amine, secondary amine and tertiary amine with a molar ratio of ca. 1:2:1 determined from ¹³C NMR spectrum (see supplementary information). On the basis of the previous work, the reactivity sequence of three types of amine should be primary amine > secondary amine > tertiary amine. Therefore the carbamate was formed via the reaction between the primary amines of PEI and CO₂ as described in Figure 1. Bubbling CO₂ into water produced no UV absorption and photoluminescence, hence the carbamate formed is the fluorophore of the photoluminescence observed.

As shown in Figure 2C, both UV absorption peak intensity and photoluminescence intensity from the aqueous solution of PEI bubbled with CO₂ change with the keeping time after CO₂ bubbling. The peak intensity of UV absorption at 364 nm increases with the keeping time before reaching the maximum after being kept for 15 days and then decrease during the observation up to 90 days. Similarly the photoluminescence intensity at 470 nm increases before reaching the maximum after being kept for 21 days and then decreases. Meanwhile Figure 2D shows the effect of the keeping time on the concentration of the carbamato anion and the dissolved CO₂ which were determined from ¹³C NMR spectra (see supplementary information). The concentration of carbamato anion reaches the maximum after being kept for 21 days and then decreases. When the concentration of carbamato anion reaches the maximum, the molar ratio of carbamato anion to the EI unit is 23 : 100, so the carbamato anion is still formed via the reaction between the primary amine of PEI and the dissolved CO₂. Meanwhile the concentration of dissolved CO₂ decreases continuously after CO₂ bubbling until being undetectable at 21 days. The consistent reducing concentration of the dissolved CO₂ should be due to the reaction with amine and evaporation into the environment. When the dissolved CO₂ concentration decreases to a certain level, the amount of carbamate starts to decrease due to decarboxylation. Figures 2C and 2D show that there is a coherent relationship between the photoluminescence intensity and the concentration of carbamato anion which further confirms that carbamato anion is the fluorophore of the photoluminescence observed.

Also Figure 2C reflects that there is a discrepancy between the effect of keeping time on the UV absorption intensity and the photoluminescence intensity. While the maximum of UV absorption is obtained after being kept for 15 days, the photoluminescence intensity reaches the highest value at 21 days. These indicate that there are more than one type of fluorophore contributing to UV absorbance and photoluminescence; otherwise a unanimous variation tendency should be observed. The other types of fluorophore should be the intermediates of carbamato anion formed when the dissolved CO₂ was still presented. However no corresponding peaks can be observed in Figure 2B(b) even though the ¹³C NMR spectrum was collected for 3 days due to a high ratio of noise to signal caused by the low concentration of PEI. In order to reduce the ratio of noise to signal, the concentration of PEI was increased by around 100 times to containing 4.6 M EI unit. Figure 3A(a) shows the ¹³C NMR spectrum of the solution of PEI in D₂O containing 4.6 M EI unit being kept for 15 days after CO₂ bubbling (also formation of HCO₃⁻ under this condition is negligible indicated by the insignificant effect of adding Ba(OH)₂ on the ¹³C NMR spectrum.). In comparison with Figure 2B(b), a new broad peak appears at 164 ppm. This peak is ascribed to the loosely-bound encounter complex rather than zwitterions because the down-field shifting of the peaks attributed to carbon in the zwitterions should be more due to the positively charged nitrogen. The loosely-bound encounter complex with a different bound degree results in the broad peak in the ¹³C NMR spectrum of D₂O bubbled with CO₂ as shown in Figure 2B(a). The peak at 125 ppm is attributed to the dissolved CO₂, the only peak observed in ¹³C NMR spectrum of D₂O bubbled with CO₂. The peak attributed to the carbamato anion appears at 160 ppm, and the molar ratio of carbamato anion to the EI unit is determined to be ca. 7.1 : 100 (see supplementary information). In a contrast experiment, Ba(OH)₂ was added to check whether HCO₃⁻ was formed based on that BaCO₃ is insoluble and barium carbamate is soluble. The addition of Ba(OH)₂ showed almost no effect on ¹³C NMR spectrum, so the amount of HCO₃⁻ formed is negligible. This is reasonable because the kinetic reaction constant of carbamate formation is five orders of magnitude higher than that of HCO₃⁻ formation. There are three types of amine in PEI, i.e., primary amine, secondary amine and tertiary amine with a molar ratio of ca. 1:2:1 determined from ¹³C NMR spectrum (see supplementary information). On the basis of the previous work, the reactivity sequence of three types of amine should be primary amine > secondary amine > tertiary amine. Therefore the carbamate was formed via the reaction between the primary amines of PEI and CO₂ as described in Figure 1. Bubbling CO₂ into water produced no UV absorption and photoluminescence, hence the carbamate formed is the fluorophore of the photoluminescence observed.
We found that thermal treatment could change the equilibrium between carbamato anion and the loosely-bound encounter complex; therefore thermal treatment was applied to differentiate the photoluminescent behaviors of carbamato anion and the loosely-bound encounter complex. Figure 3A shows 13C NMR spectrum of the solution of PEI in D2O containing 4.6 M EI unit being kept for 15 days after CO2 bubbling before and after thermal treatment at 60°C for 3 h under N2. The molar ratio of the loosely-bound encounter complex to carbamato anion is shown to increase from 1.76 to 1.94 with the molar ratio of the total carbamate to the EI unit being kept at ca. 35:100 (see supplementary information). So the thermal treatment shifts the equilibrium towards the formation of the loosely-bound encounter complex (the degradation of carbamate to recover amines and remove CO2 occurs at 100°C above33). Accordingly, the effects of thermal treatment at 60°C for 3 h under N2 on the UV absorption and photoluminescence of aqueous solution of PEI containing 50 mM EI unit being kept for 15 days after CO2 bubbling was investigated. As shown in Figure 3B, the thermal treatment reduces photoluminescence intensity at 470 nm from 1.75 a.u. to 0.17 a.u. but increases the UV absorption at 364 nm from 0.8 a.u. to 1.8 a.u. Hence the loosely binding of H3O to carbamato anion results in a higher UV absorption but a lower photoluminescence emission.

Discussion
As shown in Figure 4, there are two 3- atoms (either N, C and O or 2 O and C) containing resonance structures, i.e., A and B, for carbamato anion, without instable π-conjugated 4-atoms containing (N, C and 2 O) resonance structures with one anti-bonding orbital being filled with electrons42–47. Accordingly, there are two resonance structures for the loosely-bound encounter complex, i.e., A’ and B’. Among these resonance structures, A and A’ are the major components because O atom is more electronegative than N atom. However, n → π* transition should only occur in B and B’ with n electron from the negatively charged O atom jumping to the conjugated unit composed of N, C and O atoms, but is prohibited in A and A’ due to electronic repulsion between n electron of N atom and electrons on the carboxylate ion. Hence B and B’ are the dominant fluorophores. The n → π* transition of B and B’ under UV irradiation is supported by the red-shift in UV absorption from 364 nm
to 380 nm when water is substituted by methanol as shown in Figure 5, because $n \rightarrow \pi^*$ transition shows a red-shift and $\pi \rightarrow \pi^*$ transition shows a blue-shift reversely when solvent molecules have lower polarity and form hydrogen bonding with a lower strength\cite{14}, and methanol has a lower polarity and forms a weaker hydrogen bond with carbamate than water\cite{48,49}. In comparison with B, the loosely-bound H$_3^+$O in B facilitates the $n \rightarrow \pi^*$ transition of electron, and the H$_3^+$O in the excited state B* reduces the feasibility of relaxation of the exited electrons. These should be the cause of the stronger UV absorption but a weaker photoluminescence emission observed for carbamato anion loosely bonded with H$_3^+$O.

As shown in Figure 2A, the frequency difference between the excitation at 364 nm (wavenumber: 27472 cm$^{-1}$) and the emission at 470 nm (wavenumber: 21276 cm$^{-1}$) is 6196 cm$^{-1}$. This difference is ca. two times of the stretching vibration frequency of N-H ($v^\text{NH}$) or O-H ($v^\text{OH}$) which are ca. 3300 cm$^{-1}$ as reflected in FTIR spectrum of aqueous solution of PEI (see supplementary information Fig. S4). When H$_2$O is substituted by D$_2$O, Figure 2A(d) shows that the emission peaks shift to 442 nm (wavenumber: 22624 cm$^{-1}$), with the emission peak being kept at 364 nm. The frequency difference between excitation at 364 nm (wavenumber: 27472 cm$^{-1}$) and the emission peaks are 4848 cm$^{-1}$ which are close to two time of the vibration frequency of N-D ($v^\text{ND}$) or O-D ($v^\text{OD}$) of ca. 2400 cm$^{-1}$ as shown in FTIR (see supplementary information Fig. S4). Further, Figure 6 shows a typical excitation wavelength-depend photoluminescence profile, which was obtained from an aqueous solution of PEI containing 50 mM EI unit being kept for 10 days after CO$_2$ bubbling. The photoluminescence emission wavelength depends on the solvent type and the excitation wavelength, and the nonradiative process is via stretching vibration of the N-H in the carbamato anion. Considering the common existence of CO$_2$, the reaction with CO$_2$ should be one of the causes of photoluminescence observed from amino-containing polymers.

**Methods**

**Materials.** Hyperbranched Poly(ethylenimine) (PEI) (M$_n$: 600), butylamine, barium hydroxide, methanol, and deuterium oxide (D$_2$O) were purchased from Aldrich and used as received.

**Characterization techniques.** $^{13}$C NMR spectra were recorded on a Bruker ACF 400 FT-NMR spectrometer. The absorption and photoluminescence spectrum measurements were conducted on a Shimadzu UV-1601 PC UV-Vis spectrophotometer and a Perkin-Elmer Instrument LS 55 luminescence spectrophotometer, respectively. In order to get comparable photoluminescence intensity, all experimental parameters were kept fixed for all emission scans. FT-IR spectra of PEI solution on CaF$_2$ plate were collected in a frequency range of 1000–4000 cm$^{-1}$ with 2 cm$^{-1}$ resolution and 64 scans on a FTIR spectrometer Spectrum 2000 (Perkin Elmer) in a transmission mode.

**Exposed PEI to CO$_2$.** For UV-Vis absorbance and photoluminescence scans, CO$_2$ was introduced to a solution of PEI containing 50 mM EI in 10 mL of water or methanol in a 20 mL vial at a flow rate of $\sim$ 3 ml/min for 3 h using a one needle-inlet and one needle-outlet configuration. Then the solution was kept for a designed time before measurements. For $^{13}$C NMR experiments, D$_2$O was used instead and the concentration of EI unit was 50 mM and 4.6 M, respectively.

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![Figure 6](https://example.com/figure6.png)  
**Figure 6 | Photoluminescence emission spectra of an aqueous solution of PEI containing 50 mM EI unit being kept for 10 days after CO$_2$ bubbling being excited at (a) 330 nm; (b) 340 nm; (c) 350 nm; and (d) 360 nm.**
24. Yang, W., Pan, C. Y., Luo, M. D. & Zhang, H. B. Fluorescent Mannose-
23. Yang, W. & Pan, C. Y. Synthesis and Fluorescent Properties of Biodegradable

SCIENTIFIC
REPORTS

28. Pastor-Perez, L., Chen, Y., Shen, Z., Lahoz, A. & Stiriba, S. E. Unprecedented blue
27. Wu, D. C., Liu, Y., He, C. B. & Goh, S. H. Blue photoluminescence from
26. Shen, Y. Q.
25. Shi, Y. M. & Dong, Z. P. Fluorescent Polymeric Dendrimers and Their Applications.
24. Yang, W. & Pan, C. Y. Synthesis and Fluorescent Properties of Biodegradable
23. Yang, W. & Pan, C. Y. Synthesis and Fluorescent Properties of Biodegradable
22. Zhang, T. J. & Harper, W. A. Biodegradable Fluorescent Polymers: A Review
21. Tsai, Y. J., Hu, C. C., Chu, C. C. & Toyoko, I. Intrinsically Fluorescent PAMAM
20. Lin, S. Y.
19. Xiao, J. W., Liu, J. L., Wang, Z. Y., Zhang, H., Wang, J. & Yeh, S. C. Intrinsic Photoluminescence from Hyperbranched and Linear Polyethylenimines: Improvement of Intrinsic Photoluminescence and OLED Properties of pi-Extended Ladder-Type Heteroacenes
18. Jasmine, M. J. & Prasad, E. Fractal Growth of PAMAM Dendrimer Aggregates and
17. Jasmine, M. J. & Prasad, E. Fractal Growth of PAMAM Dendrimer Aggregates and
16. Chen, Y. et al. Photoluminescent Hyperbranched Poly(amido amine) Containing beta-Carboxyethyl and a Nonviral Gene Delivery Vector. Biomacromolecules. 22, 1162–1170 (2011).
15. Oregan, B. & Gratzel, M. A Low-Cost, High-Efficiency Solar Cell—Based on Dye-Sensitized Colloidal TiO2 Films. Nature 353, 737–740 (1991).
14. Bernard, V. Molecular Fluorescence: Principles and Applications (Wiley-VCH Verlag GmbH, Weinheim (Federal Republic of Germany), 2001).
13. Itoh, T. Fluorescence and Phosphorescence from Higher Excited States of Organic Molecules. Chem. Rev. 112, 4541–4568 (2012).
12. Franchini, R. & Marzolla, M. Polymer Chemistry and Properties (CRC Press INC, 2010).
11. Hansen, C. M. Hansen Solubility Parameters: A User’s Handbook (CRC Press, 2010).
10. Le Grand, M. J. & O’Connor, P. J. The Physical Properties of Polymers, 2nd edn (Artech House, 1981).
9. Hampe, E. M. & Rudkevich, D. M. Reversible covalent chemistry of CO2. Angew. Chem. Int. Ed. 41, 9306–9313 (2002).
8. Liu, Y. A. Porphyrin-Sensitized Solar Cells with Cobalt (II/III)-Based Redox Electrolyte Exceed 12 Percent Efficiency. Science 334, 629–634 (2011).
7. Fritsch, T. Fluorescence and Phosphorescence from Higher Excited States of Organic Molecules. Chem. Rev. 112, 4541–4568 (2012).
6. Kim, M. J. et al. Tuning of spacer groups in organic dyes for efficient inhibition of charge recombination in dye-sensitized solar cells. Dyes Pigm. 95, 134–141 (2012).
5. Liu, Y. et al. Fluorescent Chemosensor for Detection and Quantitation of Carbon Dioxide Gas. J. Am. Chem. Soc. 132, 13951–13953 (2010).
4. Liu, Y. et al. Specific Detection of D-Glucose by a Tetraphenylethylene-Based Fluorescent Sensor. J. Am. Chem. Soc. 133, 660–663 (2011).
3. Jasmine, M. J. & Prasad, E. Fractal Growth of PAMAM Dendrimer Aggregates and Its Impact on the Intrinsic Emission Properties. J. Phys. Chem. B 114, 7735–7742 (2010).
2. Tsien, R. Y. The green fluorescent protein. Annu. Rev. Biochem. 67, 509–544 (1998).
1. Shaner, N. C., Steinbach, P. A. & Tsien, R. Y. A guide to choosing fluorescent proteins. Nat. Methods 2, 905–909 (2005).

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Author contributions
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