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Highly stable dendritic trityl radicals as oxygen and pH probe†

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Abstract

Novel dendritic trityl radicals (DTR1 and DTR2) with a TAM radical core, PAMAM branching and carboxylate exterior surface exhibit high stability towards oxidoreductants as evidenced by their electrochemical and EPR properties, offering potential application as dual oxygen and pH probe.

There has been great interest in the development of tetrathia-triarylmethyl (TAM) radicals, a class of trityl radicals, since these compounds have found wide application in electron paramagnetic resonance (EPR) spectroscopy and imaging.[1] Due to the stability as well as the narrow EPR line width of TAM radicals under physiological conditions, they provide high sensitivity and resolution for the measurement of oxygen concentrations, redox status and reactive oxygen species (ROS) in biological systems.2 However, their stability has been problematic when exposed to biological oxidoreductants,3 whose generation is often due to changes in cellular oxygen level,4 thus limiting their application in the investigation of ROS-mediated diseases.5 Although TAM radicals have been proposed for pH measurement,[6] these radicals are prone to self-aggregation at acidic pH resulting in broad and weak EPR signals thereby decreasing their EPR sensitivity and limiting their potential for biomedical imaging applications.[2b] Therefore, there is a pressing need to develop new trityl radicals with improved properties in aqueous solution.

Dendrimers with highly symmetric and branched architecture are especially useful for covalent encapsulation of active core moieties.[7] Dendritic encapsulation changes the thermodynamic properties of these cores and builds kinetic barriers, resulting in their stabilization.[8] Moreover, encapsulation has been shown to prevent self-aggregation of dye molecules,[9] or led to the isolation of reactive functional molecules from biomolecules in *in vivo* systems, thereby reducing their toxicity.[10]

Herein, we report the first example of dendritic trityl radicals (Chart 1), in which the TAM radical **CT-03** is used as a core for the synthesis of first (**DTR1**) and second (**DTR2**) generation PAMAM dendrimer[11] with carboxylate groups on the exterior surface. Electrochemical properties, stability towards biological oxidoreductants, and non-aggregation under acidic conditions were investigated. Moreover, the potential use of a **DTR2**-Cu^{2+} complex as pH probe was also explored.

†Electronic supplementary information (ESI) available: Details of synthesis and spectroscopic characterization.

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The synthesis of **DTR1** and **DTR2** is described in the ESI.† As expected, each EPR spectrum of **DTR1** and **DTR2** exhibits a singlet signal (Fig. 1). Almost identical linewidths were observed for **DTR1** (826 mG) and **DTR2** (812 mG) suggesting that increasing dendrimer generation has only a minor effect on their linewidths and spin relaxation. These broader linewidths for **DTR1** and **DTR2** relative to **CT-03** (190 mG) could be partly due to the presence of resolved or unresolved hyperfine splittings from the paramagnetic nitrogen and hydrogen nuclei of the three amide groups that are directly linked to the aromatic rings.

Fig. 2 shows the cyclic voltammograms of the TAM radicals. **CT-03** underwent one-electron quasi-reversible reduction at -0.63 V *vs*. Ag/AgCl to the corresponding trityl anion and one-electron reversible oxidation at 0.45 V *vs*. Ag/AgCl to the cation form. However, the voltammetric waves observed for **CT-03** disappeared in both **DTR1** and **DTR2**. These changes in electrochemical properties indicate a decrease in the electron-transfer rates between the buried TAM radical and the electrode surface due to dendritic shielding of the radical moiety. This protection is unusual since in most cases[12] the disappearance of the voltammetric waves were observed from second or higher generation dendrimers. The highly negatively charged surface and hydrophobic interior could have a significant effect on the electron transfer rate from the electrode to the radical core.

In order to further assess the effects of dendritic shielding on the TAM radicals, their stability towards various biological oxidoreductants was investigated (Table 1). **DTR1** and **DTR2** have similar stability to **CT-03** towards reducing agents such as glutathione (GSH) and ascorbate (Asc), but also exhibit a much higher stability towards several oxidants. For example, while the paramagnetism of **CT-03** was quenched to different degrees by $ROO^{\cdot}$, $O_2^{\cdot-}$, $^{\cdot}CH_3$ and $HO^{\cdot}$, **DTR1** and **DTR2** showed high stability in the presence of the same amount of these radical sources with almost no quenching. Dendritic encapsulation, therefore, provides marked protection of the TAM radicals.

Acidic titration reveals that the EPR spectral linewidth of **DTR1** and **DTR2** remained constant over the pH range of 2.0-7.8 (see ESI†), but for **CT-03**, the linewidth broadening was observed as the pH was decreased, due to self-aggregation.[2b] Thus, it can be inferred that the dendritic branches could increase the solubility of **DTR1** and **DTR2** in acidic conditions, thereby preventing self-aggregation. The non-aggregating property of the dendritic trityls was further confirmed by the UV-Vis spectroscopy (see ESI†).

The potential application of the dendritic radicals as oxygen sensors was explored. **DTR2** and **DTR1** showed nearly identical oxygen-dependent EPR line broadening response but relatively lower than that of **CT-03** (see ESI†). This lower sensitivity is possibly due to the presence of broader linewidths in anaerobic solution but also partly due to the reduced accessibility of the radical center to the oxygen molecule.[2c] However, the non-aggregation makes the dendritic trityls well-suited for oxygen measurement at acidic pH and would be very useful for the imaging of ischemic tissues and the stomach.

The acid-induced release of Cu^{2+} from the **DTR2**-Cu^{2+} complex was exploited as a means to measure pH by EPR. Similar studies on other dendrimers have been previously reported[13] but used spectrophotometric techniques. The EPR signal of **DTR2** (10 μM) showed a sharp decrease upon addition of Cu^{2+} and completely disappeared at the [Cu^{2+}]/[**DTR2**] ratio of 2 (see ESI†) due to strong spin-spin interaction between the radical and the bound Cu^{2+}. Interestingly, the EPR signal can be regenerated from the above conditions after addition of HCl with an increase in signal intensity as the pH decreases (Fig. 3A). The intensity as a function of pH is shown in Fig. 3B. At neutral pH, Cu^{2+} binds to one or more tertiary amines

†Electronic supplementary information (ESI) available: Details of synthesis and spectroscopic characterization.

of the dendritic branch.[14] The protonation of the tertiary amines occurs from 6.5 to 5.0, and leads to the binding of Cu^{2+} exclusively with carboxylate-O.[14] Since Cu^{2+} is farther away from the central radical at this point, a slight increase in the signal is observed. Further acidification of the solution from pH ~5.0 to ~3.8 leads to the release of Cu^{2+} bound by the carboxylate groups as shown by the dramatic increase of the EPR signal. With decrease of pH below 3.8, only a slight enhancement of the EPR signal was observed due mostly to the release of Cu^{2+} from its non-selective absorption. Therefore, the pH-dependent EPR signal enhancement from the **DTR2**-Cu^{2+} complex can be exploited to measure pH. It provides by far the most sensitive and stable EPR probe known for measurement of pH and is suitable for biomedical spectroscopy and imaging.

Of note, while this manuscript was being processed, novel amino derivatives of trityl radicals were also reported to function as dual pH and oxygen probes.[15] These radicals have EPR spectra with complex hyperfine structure which is directly altered by pH. However, their complex spectra may limit sensitivity and ease of use in EPR imaging applications.

In summary, the first example of dendritic TAM radicals was synthesized and characterized. Covalent encapsulation by the PAMAM dendrimer is highly effective in enhancing their stability and improving their solubility over a wide pH range. Importantly, the **DTR2**-Cu^{2+} complex is shown to be a highly effective pH probe. These new molecules and their potential for derivatization offer the possibility of new biomedical applications as probes for molecular imaging and site specific oximetry, and pH measurements under both normal and extreme conditions such as highly acidic and reducing/oxidizing environments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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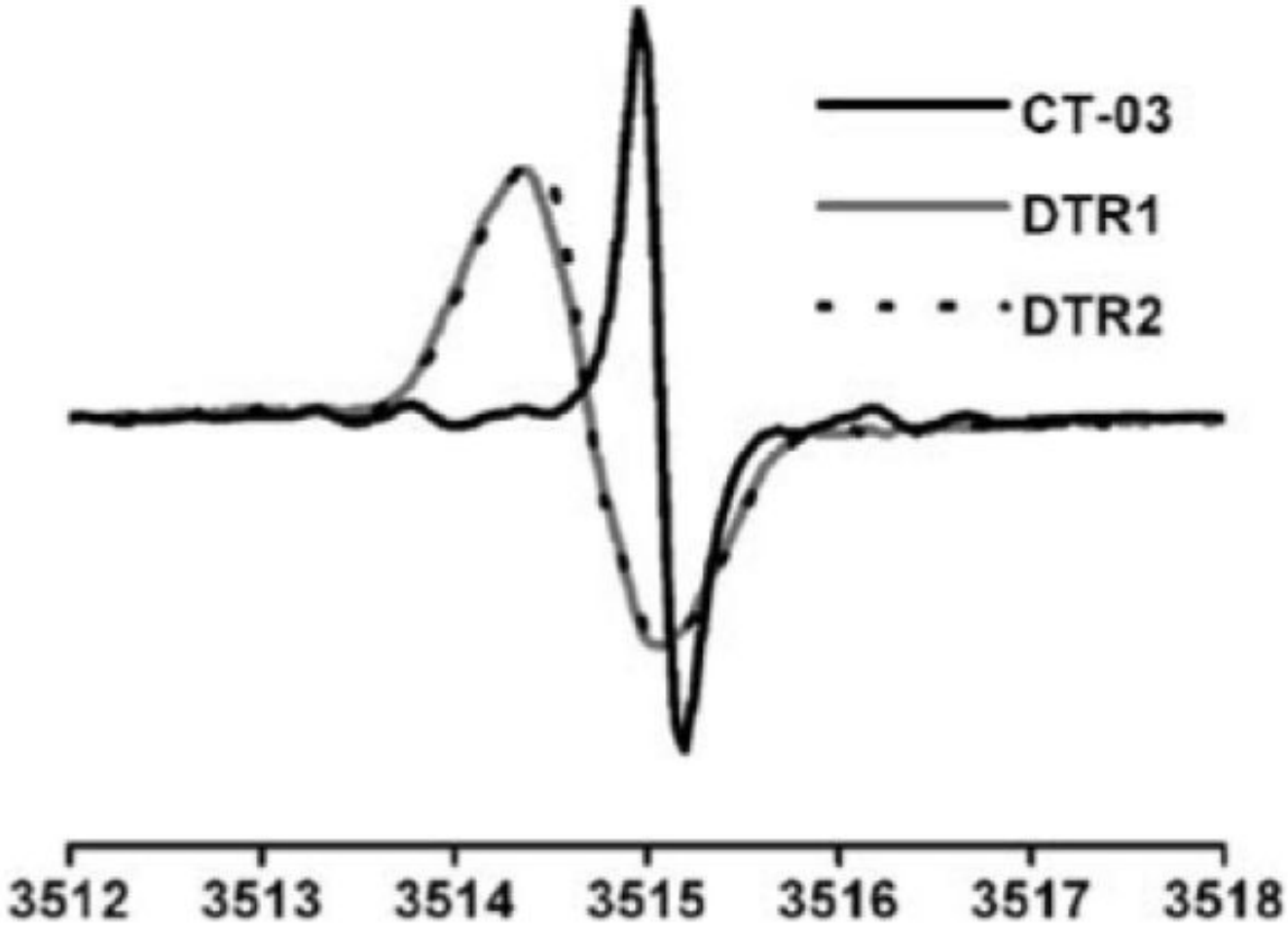

Fig. 1.
X-Band EPR spectra of aerobic aqueous solutions of **DTR1, DTR2** and **CT-03**. The linewidths are 826, 812 and 190 mG for **DTR1, DTR2** and **CT-03**, respectively.

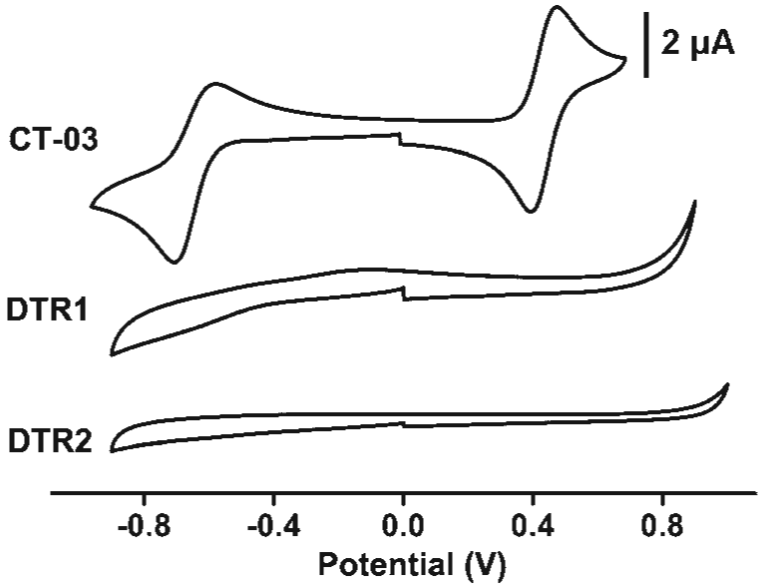

Fig. 2.

Cyclic voltammograms of **CT-03, DTR1** and **DTR2** in phosphate buffer solution (PBS) (0.1 M, pH 7.4) containing 0.1 M NaCl; scan rate, 50 mV s^{-1}.

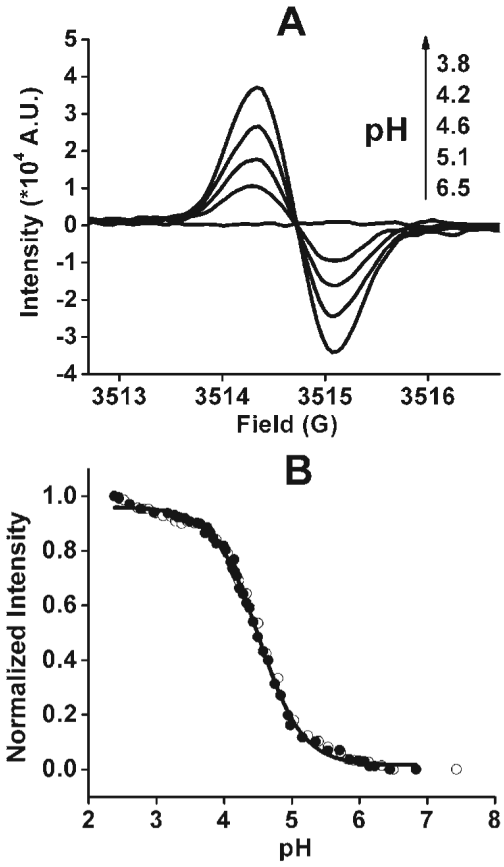

Fig. 3.

pH dependence of the EPR spectra (A) and signal intensity (B) of the complex of **DTR2** (10 μM) with Cu^{2+} (25 μM). In Fig. B, titration was carried out from alkaline to acidic pH (●) and acidic to alkaline pH (○), respectively.

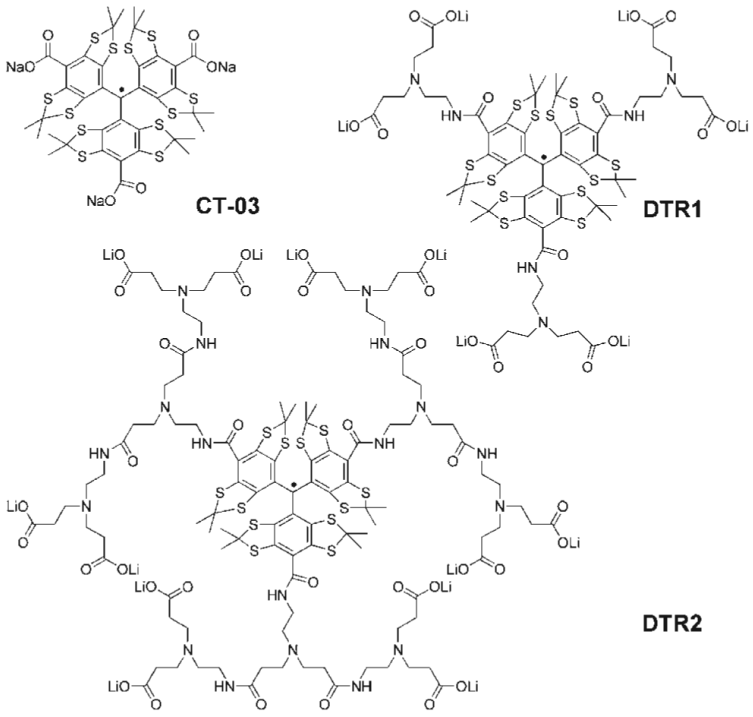

Chart 1.
Molecular structure of dendritic trityl radicals.

Table 1

Percentage of **CT-03, DTR1** and **DTR2** remaining after exposure to various reactive species in PBS[a]

Trityl	Asc	GSH	$ROO^{\cdot}$	$O_2^{\cdot-}$	$^{\cdot}CH_3$	$HO^{\cdot}$	H_2O_2
CT-03	96.7	94.6	59.7	55.2	86.5	90.5	97.1
DTR1	99.2	100	99.4	97.8	96.6	99.7	98.8
DTR2	99.3	99.9	99.7	99.5	99.7	100	100

[a]EPR spectra were recorded 30 min after the radical production was initiated in the presence of trityl (10 μM) in PBS. The percentage was obtained by dividing spectral intensity of each sample by that of the control consisting of the trityl alone. Each value was the average of triplicate measurements. See more details in the ESI.†