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THERMAL DECOMPOSITION OF A GENERATION 4.5 PAMAM DENDRIMER PLATINUM DRUG CONJUGATE

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A nanoscale multivalent platinum drug based on a poly(amidoamine) [PAMAM] dendrimer (generation 4.5, carboxylate surface) has been synthesized and fully characterized using a variety of spectroscopic, chromatographic and thermal methods. Treatment of the dendrimer with an aqueous solution containing an excess diaquo(*cis*-1,2-diaminocyclohexane)platinum(II) produces a conjugate containing approximately forty (diaminocyclohexane)platinum(II) moieties at the surface of the dendrimer. This material undergoes smooth two-stage thermal decomposition to provide residual platinum oxide reflecting the platinum loading in the drug.

Keywords: *dendrimer-based organoplatinum drugs, drug delivery, polymeric drugs, thermal decomposition, time-release drugs*

Introduction

Recently, there has been considerable interest in hydrophilic, biocompatible polymeric materials that could serve as drug carriers to achieve site-specific and time-controlled delivery of therapeutics, thus alleviating undesired side effects and enhancing the efficacy of treatment [1–3]. Traditionally linear hydrophilic polymers have been examined for potential application as drug carriers [4–10]. The advent of dendrimers, which are highly branched macromolecules with precisely controlled size, shape and end-group functionality, has provided an excellent opportunity to design novel drug carriers. PAMAM dendrimers are the first complete dendrimer family to be synthesized, characterized and commercialized. Biological studies using PAMAM dendrimers have demonstrated that high generation PAMAM dendrimers are non-immunogenic and display low mammalian toxicity, while anionic PAMAM dendrimers (surface groups with carboxylic or hydroxylic functionalities) are non-toxic in vitro [11, 12]. Platinum complexes exhibit strong antitumor activities, especially the well known cisplatin [*cis*-diamminedichloroplatinum(II)] and carboplatin [*cis*-diammine(cyclobutanedicarboxylato)platinum(II)] widely used for the treatment of human testicular, overian, bladder, head and neck carcinomas. However, the side-effects such as nephrotoxicity and myelotoxicity are major drawbacks of these compounds for clinical applications. Molecular simulation studies have suggested that higher generation PAMAM dendrimers ($G \geq 4$) are dense-packed spheroids [13]. These observations suggest that a dendrimer of appropriate size should function as a well-behaved carrier for platinum species which display therapeutic properties. Coordination of an appropriate platinum moiety to the dendrimer surface would generate a drug formulation with water solubility, dosage limitations, and response characteristics superior to those of classical platinum drugs.

Experimental

Materials

Carboxylate-terminated PAMAM (G4.5) dendrimer having an ethylenediamine core was purchased as 10–15% methanol solution (Dendritic Nanotechnologies, Inc., Mt. Pleasant, MI). Prior to use, the methanol was removed under reduced pressure at room temperature and then the pH of an aqueous PAMAM (G4.5) solution was adjusted to 4.0 by adding dilute aqueous nitric acid solution.

Potassium tetrachloroplatinate, *cis*-1,2-diaminocyclohexane, and silver nitrate were purchased from Aldrich Chemical Co. and used as received. Dialysis cassettes having a molecular mass cutoff of 3500 were obtained from Pierce Biotechnology Co. A benchtop pH meter (model IQ240) was purchased from I.Q. Scientific Instruments and calibrated each time before use. In general, synthesis reactions were performed under a static atmosphere of prepurified nitrogen. The complex of [(DACH)$PtCl_2$] (DACH=1,2-diaminocyclohexane) was prepared as previously reported [10]. The PAMAM (G4.5)–Pt conjugate was synthesized through the metathesis of intermediate [(DACH)Pt$(OH_2)_2$] with PAMAM (G4.5) and further purified by dialysis against deionized water.

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Polyacrylamide gels (20%) were prepared according to a standard protocol [14]. Gels were run between 70 and 80 V for 2–3 h. The sample buffer was 0.25% bromophenol blue and 40% (*w*/*v*) sucrose in water. Samples were prepared by adding 0.1 volume of running buffer to each dendrimer and conjugate solution. The running buffer was TBE (90 mM *tris*-borate, 2 mM EDTA). Ethidium bromide and Coomassie brilliant blue R250 were used for staining and visualization.

Methods

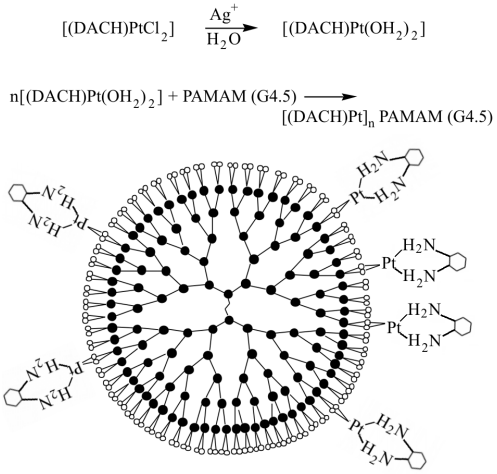
The resulting sample was checked for purity using thin layer chromatography and then the platinum content was determined by a number of techniques. Nuclear magnetic resonance (NMR) spectra (^{1}H and ^{13}C) were recorded using a Varian Mercury Plus 300 MHz NMR Spectrometer. The sample was initially dried by lyophilization and then redissolved in deuterium oxide to generate a solution (0.5–2.0%) suitable for analysis. All NMR chemical shifts were reported in parts per million (δ) relative to internal tetramethylsilane (TMS δ=0.00). MALDI-TOF mass spectra were acquired in positive- and negative-ion mode using a Bruker Daltonics flexAnalysis instrument equipped with a pulsed nitrogen laser emitting at 337 nm. An overlayer preparation was used with a dihydroxybenzoic acid (DHB) matrix. Samples were analyzed in linear mode using a delayed extraction time of 550 ns and an accelerating voltage of 20 kV. The laser light intensity was adjusted to provide the optimal signal-to-noise ratio. All spectra were the result of averaging 200–300 laser shots.

Thermogravimetry was accomplished using a TA Instruments, model 2950 TGA instrument interfaced with the Thermal Analyst 2100 control unit at a heating rate of 10°C min^{-1}. The sample (5–10 mg) was contained in a platinum sample pan. TG cell was swept with nitrogen at 50 mL min^{-1} during degradation runs.

Results and discussion

To generate a well-defined dendrimer-drug conjugate, a generation 4.5 poly(amidoamine) [PAMAM] dendrimer was selected as substrate. For this purpose, the dendrimer has several positive features. As noted above, there is a close-packing of functional groups at the surface, namely 128 carboxylate groups in the case of PAMAM (G4.5). This makes it unlikely that interaction with a platinum species would occur anywhere other than the surface. In addition, the carboxyl functionality should serve as labile ligands for platinum moieties such that release of active platinum species should occur at a sustained rate over a period of time. The (*cis*-1,2-diaminocyclohexane)platinum(II) moiety was selected as the platinum component of the dendrimer–platinum conjugate since 1,2-diaminocyclohexane is known to serve as a superior inert ligand for the preparation of platinum antitumor compounds. [(DACH)PtCl$_2$] was prepared from tetrachloroplatinate as previously described [10]. This, in turn, was treated with aqueous silver nitrate to generate the corresponding diaquo species. Treatment of this intermediate with a solution of PAMAM (G4.5) dendrimer in water produced the dendrimer-based platinum drug with carboxylate groups as the labile ligands at the surface of dendrimer, as presented in Scheme 1.

The dendrimer-platinum conjugate generated in this way was characterized by NMR, MALDI-TOF MS and TG. That complexation had occurred is readily apparent from the downfield chemical shift of adsorption due to the carboxylate group in the ^{13}C NMR spectrum of the PAMAM–Pt conjugate, as shown in Table 1.



Scheme 1 Synthesis of a generation 4.5 PAMAM dendrimer-(1,2-diaminocyclohexane)platinum(II) conjugate

Table 1 ^{13}C chemical shifts (δ) for PAMAM (G4.5) and its conjugate

Compound	δ, –CO–	δ, –CH_2CH_2–	δ, DACH
PAMAM (G4.5)	179.50, 175.21, 174.65	51.37, 50.42, 49.74, 48.28, 36.74, 35.64, 32.54	
PAMAM (G4.5)–(DACH)Pt	179.50, 178.52, 177.70, 173.19	53.34, 51.75, 50.94, 49.49, 34.64, 30.55, 30.40	60.67, 59.80, 58.16, 56.22, 25.54, 20.89

Table 2 Thermal decomposition of a PAMAM generation 4.5 dendrimer (1,2-diaminocyclohexane)platinum(II) conjugate

Decomposition	Mass loss/%	Degradation onset temperature/°C	Temperature for maximum degradation rate/°C
Stage 1	41.6	173.7	220.7
Stage 2	34.2	518.7	745.1

The MALDI-TOF spectrum also supports this and indicates that about 40 (DACH)Pt moieties per dendrimer are bound to the surface carboxylates. That this is a maximum possible loading of (DACH)Pt units was apparent from a series of experiments in which the ratio of [(DACH)Pt$(OH_2)_2$] to dendrimer was increased well beyond that required to load the theoretical maximum of 64 (DACH)Pt units. For example, even when the ratio of [(DACH)Pt$(OH_2)_2$] to dendrimer was 192 to 1 (well above that required to saturate the carboxylate surface), the dendrimer (DACH)Pt conjugate generated contains 40 (DACH)Pt units. The proton NMR spectrum of the dendrimer conjugate strongly supports the conclusion from MALDI-TOF analysis. Comparison of the integration of the chemical shifts for the methylene region of the dendrimer with that for the DACH ligand suggests that 42 (DACH)Pt units were present per dendrimer. This is in close agrement with the value from the MALDI-TOF MS analysis and clearly supports the observation that the maximum loading is approximately 40 (DACH)Pt moieties per dendrimer molecule. This is consistent with a recent report of a similar limitation for the interaction of PAMAM (G4, amine surface) with an organic ligand [15].

This is further substantiated by the thermal behavior of the conjugate. As may be noted from Fig. 1 this material undergoes well-behaved thermal decomposition [16]. This decomposition occurs in two major stages. The first with an onset of 173°C reflects a loss of 41.6% or the initial mass. The second with onset at 518.7°C accounts for 34.2% of the sample mass (Table 2). A stable residue of platinum oxide is obtained at 910°C. The mass of the residual oxide corresponds to 24.2% of the initial sample mass. This is in excellent agreement with that expected (24.7%) for a dendrimer conjugate containing forty (1,2-diaminocyclohexane)platinum(II) moieties.

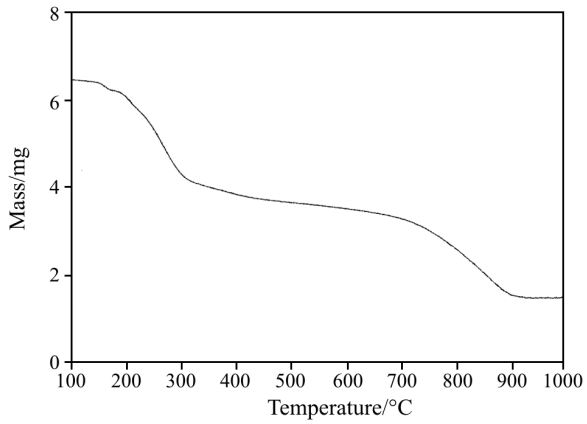


Fig. 1 TG curve for the decomposition of a generation 4.5 PAMAM dendrimer-(1,2-diaminocyclohexane)platinum(II) conjugate

Unlike previous dendrimer–platinum systems in which ill-defined platinum species were placed at random within the dendrimer, the dendrimer–platinum conjugate described here has a well-defined platinum species, (DACH)Pt(II), placed only at the surface of a PAMAM (G4.5) dendrimer. The dendrimer should function both as a carrier for the platinum moieties and as a labile ligand to release active platinum species into the extracellular fluid as a function of time in a biological system.

Conclusions

A new platinum drug in which the inert ligand is *cis*-1,2-diaminocyclohexane and the labile ligands are carboxylate groups at the surface of a PAMAM (G4.5) dendrimer has been synthesized and characterized. This drug undergoes smooth two-stage thermal decomposition to afford a residue of platinum oxide corresponding to the level of platinum present in the formulation. This corresponds to forty (1,2-diaminocyclohexane)platinum(II) moieties per dendrimer molecule.

Acknowledgements

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