Linear-Dendritic ABA Triblock Copolymers as Nanocarriers

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Received 6 December 2005; accepted 7 July 2006
DOI 10.1002/app.25583
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Linear-dendritic ABA triblock copolymers containing PEG were used for transport the small guest molecules such as 5,7-dibromo-8-hydoxy quinoline, ibuprofen, and Congo red. Nanocarriers containing guest molecules were soluble in water and in some of the organic solvents. Encapsulated guest molecules were soluble in some of the solvents, which they cannot be solved in them solely, for example, chloroform is a very poor solvent for Congo red, but encapsulated Congo red by nanocarriers is soluble in chloroform. The linear-dendritic copolymers/guest molecule complexes were stable at room temperature for about 10 months; during this time, guest molecules did not release from linear-dendritic copolymers/guest molecule complexes. The controlled release of guest molecules from linear-dendritic copolymers/guest molecule complexes *in vitro* conditions also was investigated. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 267–272, 2007

Key words: dendrimers; biocompatible; citric acid; poly (ethylene glycol); drug delivery

INTRODUCTION

The application of dendrimers in pharmaceutical and medical chemistry is fast becoming one of the most attractive areas of dendrimer chemistry. The potential of dendrimers as gene-delivery agents has been explored for several years. In 1993, Haensler and Szoka published the first report[1] of the use of PAMAM dendrimers for gene transfection. Subsequently, Szoka and coworkers reported that ''fractured dendrimers,'' obtained by heat treatment of intact dendrimers in a variety of solvolytic solvents, enhanced the transfection activity dramatically.[2] Another research group also reported the efficient transfer of genetic material using PAMAM dendrimers.[3,4] More recently, Choi et al.[5] reported a novel linear-dendritic block copolymer that formed a water-soluble complex with plasmid DNA under physiological conditions. The complex was found to take a globular shape with a relatively narrow size distribution, and this novel copolymer may be used for gene delivery. The well-defined structure, compact globular shape, size monodispersity, and controllable ''surface'' functionalities of dendrimers make them excellent candidates for evaluation as drug carriers.

Dendrimers can be used as potential drug-delivery agent in at least two ways: first, the drug molecules can be physically entrapped inside the dendritic structure; and second, the drug molecules can be covalently attached onto surface or other functionalities to afford dendrimer–drug conjugates. The internal ''cavity'' of an appropriately designed dendritic structure could be used for the entrapment of drugs with the possibility of subsequent controlled release.

Studies by several research groups[6–12] have shown that the interior of a dendrimers is capable of encapsulating guest molecules. The first strategy for the encapsulation of guest molecules in dendrimers is physical encapsulation. Meijer and coworkers reported that the guest molecules such as Rose Bengal can be physically entrapped in the internal cavity of high-generation poly(propylene imine) dendrimers when an amino acid derivative is used to cap each end group of the dendrimer.[7] Because the amino acid derivative they used for capping was bulky, sterically crowded and subjected to the hydrogen bonding, the entrapped guest molecules were not able to diffuse out of the dense packed shell of the dendrimer into the surrounding solution. However, selective liberation of the encapsulated guest molecules could be achieved, on the basis of their shape or size, by removing the shell either partially or completely using finely tuned hydrolytic conditions.[8] The second strategy for the encapsulation of guest molecules in dendrimers is based on multiple noncovalent chemical interactions, such as hydrogen bonding, between guest molecules and the dendritic structure. Newkome et al.[9] reported a dendritic host containing multiple hydrogen bonding sites at its

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Journal of Applied Polymer Science, Vol. 104, 267–272 (2007)

core. Recently, Twyman et al.[10] reported two water-soluble hydroxyl-terminated PAMAM dendrimers and studied their complexation with a variety of small acidic hydrophobic guest molecules as a model for potential use in drug-delivery applications. They found that the 1 : 1 complex of dendrimer–benzoic acid is infinitely soluble in water and stable at neutral pH, but unstable in acidic conditions. Moreover, they suggested that an interaction between the acidic proton of the model guest molecule and the basic tertiary nitrogens of the dendritic host is involved.

The third and the most easily implemented strategy for the encapsulation of guest molecules in dendrimers make use of hydrophobic interactions. Newkome et al.[11] prepared dendritic macromolecules with a hydrophobic interior and hydrophilic chain ends; these molecules were said to behave as unimolecular micelles capable of solubilizing various hydrophobic compounds in aqueous solution. Fréchet et al.[12] reported that simple, well-characterized poly (aryl ether) dendrimers bearing carboxylic groups as chain ends are able to enhance the water solubility of hydrophobic compounds.

However, several drawbacks limit transportation of small guest molecules by perfect dendrimers. Perfect dendrimers have a relatively rigid molecular structure with interior cavities, are not flexible, and they have certain dimensions for accepting guest molecules with a certain and defined size.[7,8,12–14] For encapsulation and release of guest molecules from perfect dendrimers, a protection and deprotection reaction series is necessary.[8] On the other hand, for the synthesis of perfect dendrimers, several steps of reactions and purifications are needed.

Linear-dendritic copolymers are other class of dendritic supramolecules developed by Gitsov and coworkers.[15–22] In contrast to drawbacks of perfect dendrimers, linear-dendritic polymers have flexible interiors and they can encapsulate variety of small guest molecules, they also can be synthesized by one-step reactions. Recently, it is shown that which transport capacity of some of linear dendritic copolymers is much more than perfect dendrimers. These advantages and interesting properties of linear-dendritic polymers have stimulated investigation in this area.[23–29]

Recently, we synthesized some of dendritic compounds containing citric acid and poly(ethylene glycol); also, we investigated their thermoreversible hydrogel properties and their application as the new deliverer compounds.[30]

Here, we report our attempts to preparation of some drug/dendrimer complexes containing G_1, G_2, and G_3 of dendritic citric acid–poly(ethylene glycol)-dendritic citric acid (CPEC) and some guest molecules such as 5,7-dibromo-8-hydoxy quinoline, ibuprofen, and Congo red. Also, release of guest molecules from host molecules has been investigated.

EXPERIMENTAL

Materials

5,7-Dibromo-8-hydoxy quinoline and pyridine (purified with refluxing over NaOH for 2 h and subsequent distillation) were obtained from Merck. Linear dendritic compounds were prepared according to reported paper.[30] Thionylchloride (from Merck) was purified by refluxing a mixture of 10 wt % linseed oil in thionylchloride for 2 h and subsequent distillation. Dicyclohexyl carbodiimide (DCC) and 5,7-dibromo-8-hydoxy quinoline were purchased from Merck. Ibuprofen was purchased from Aldrich.

Instrumental measurements

FTIR spectra of film cast cut of samples were recorded on a Shimadzu Model FT-IR-8101M spectrometer. ^{1}H-NMR spectra were recorded on FT-NMR (400 MHz) Brucker in DMSO-d_6, acetone deuterium and acetone in the presence of DMSO. For the investigation of bis-glubolar/drug complex compounds, UV 2100 Shimadzu spectrophotometer was applied.

Preparation of the $G_{n\ (n\ =\ 1–3)}$/5,7-dibromo-8-hydroxy quinoline complexes

For preparation of $G_{n\ (n\ =\ 1–3)}$/5,7-dibromo-8-hydroxy quinoline complexes, a solution of dendrimers (0.1 g) in 20 mL THF were added to a round-bottom flask equipped with reflux condenser and a magnetic stirrer. A solution of drug (1 g in 20 mL THF) was added to dendrimer solution, and then it was stirred for 2 h at 30°C. Complexes were precipitated in *n*-hexane. The precipitated complex was dissolved in dichloromethane, and then it was precipitated in diethylether to yield a green light compounds.

IR: cm^{-1}, COOH (3520–2665), C=O (1750–1732), C=C (1625, 1580, 1490, 1450), C—O (1120–1215).

Preparation of the $G_{n\ (n\ =\ 1–3)}$/ibuprofen, complexes

A solution of dendrimers (0.1 g in 20 mL ethanol/DMF (3 : 1 v/v)) was added to a solution of ibuprofen (1 g in 20 mL ethanol/DMF). This mixture was stirred for 2 h at 30–50°C. Then, the complexes were precipitated in diethylether. The precipitated complexes were dissolved in dichloromethane and reprecipitated in diethylether to yield a yellow compound.

IR: cm^{-1}, COOH (3447–2750), C=O (1710–1749), C=C (1500, 1480), C—O (1111, 1190).

Preparation of the $G_{n\ (n\ =\ 1–3)}$/Congo red, complexes

Congo red (1 g) was added to a solution of dendrimers (0.1 g in 10 mL chloroform). The mixture

was stirred for 2 h at room temperature, and then it was left for 1 day at room temperature. Then, the mixture was filtered by microfilters and a clear solution was obtained.

IR: cm^{-1}, COOH (3520–2665), C=O (1750–1732), C=C (1600, 1480), C—O (1120–1215).

General procedures for release of drug from dendrimer/drug complexes

The dendrimer/drug complexes were dried in vacuum at room temperature. The released drugs from the dendrimer/drug complexes were measured at 37°C using a cellophane membrane dialysis bag. The dendrimer/drug complexes (70 mg) were poured in 5 mL aqueous buffered solution (pH 1, 7.4, and 10). The mixture was conducted into a cellophane membrane dialysis bag, and the cellophane bag was closed and transferred into a flask containing 25 mL of the same buffer solution maintained at 37°C. The bulk solution was stirred continuously at 800 rpm, and a sample solution (3 mL) was withdrawn at regular selected intervals and replaced with equal volumes of the media. The quantity of released drug was analyzed by means of a UV spectrophotometer and determined from the calibration curve obtained previously under the same conditions. The amount of released drugs was determined by a 2100 Shimadzu UV spectrophotometer at the absorption maximum of the free drug using a 1-cm quartz cell.

RESULTS AND DISCUSSION

Three generation of linear-dendritic copolymers were used for delivery of both hydrophobic and hydrophilic guest molecules. Host–guest systems were soluble in aqueous solution.

Host–guest systems were prepared by the addition an extra amount of drug to a solution of linear-dendritic copolymers in a suitable solvent. Nontrapped drug was separated from host–guest systems by precipitation of linear-dendritic copolymers in a suitable nonsolvent. Purified compounds were dried under vacuum.

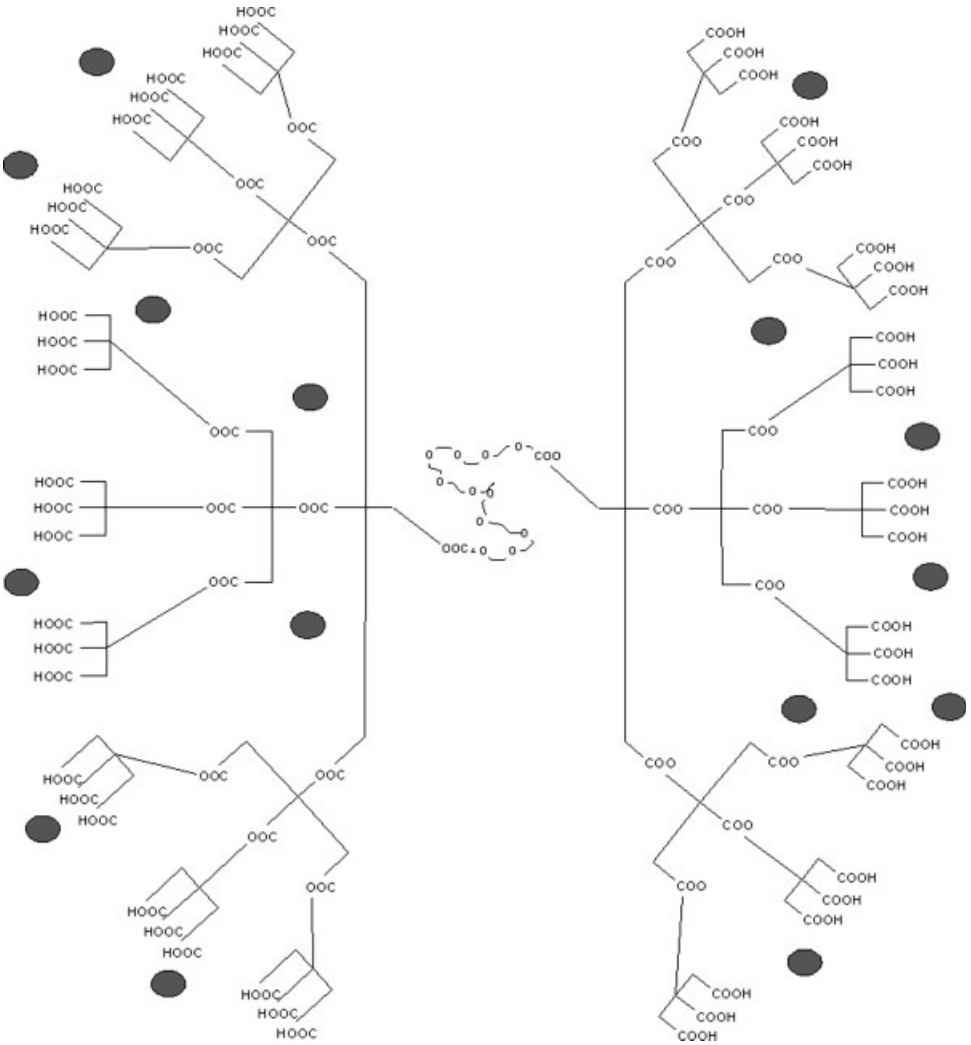
Three different small guest molecules were chosen to transport by linear-dendritic copolymers. Solubility properties of guest molecules were different: Congo-red is a hydrophilic compound, but ibuprofen and 5,7-dibromo-8-hydroxy quinoline are hydrophobic compounds. Encapsulation the different guest molecules with different solubility by linear-dendritic copolymers prove capability of nanocarriers to encapsulation and salvation variety of hydrophobic and hydrophilic small guest molecules in different solvent. Ibuprofen is a well-known drug, but two other guest molecules are not drug and are considered as model drugs. 5,7-Dibromo-8-hydroxy quinoline and Congo red contain nitrogen heteroatom, but ibuprofen does not contain nitrogen heteroatom; hence, it is possible to understand the role of heteroatom and polarity on transport capacity and rate of release (Scheme 1).

Figure 1 displays the image of trapped Congo red by dendrimers in chloroform solution; red color of chloroform solution is related to the encapsulated Congo red by dendrimers, because chloroform solution of dendrimers is colorless and solubility of Congo red in chloroform is very low. On the other hand, increasing the red color of solutions with increased generation (from G_1 to G_3) proves increasing the amount of trapped Congo red with increased generation visually.

Intensity of λ_{max} of encapsulated guest molecules by linear-dendritic copolymers increases when the generation of linear-dendritic copolymers increases; hence, it can be assumed that higher generation of linear-dendritic copolymers encapsulated higher amount of guest molecule. Figure 2 shows the UV spectra of trapped Congo red by different generations of dendrimers.

Amount of trapped guest molecule was measured by UV and NMR spectra. Mole percentage of trapped guest molecule increases with increasing the generation of linear-dendritic copolymers (Table I). Maximum and minimum amount of trapped guest molecule is related to 5,7-dibromo-8-hydroxyqunoline and ibuprofen, respectively. On the other hand, mole percentage loading of Congo red guest molecule is between 5,7-dibromo-8-hydroxyqunoline and ibuprofen.

Amount of loaded guest molecules by host molecule depends on several factors, such as the solubility of the guest molecules in the surrounding medium (hydrophilic/hydrophobic), the size of the micellar core (monodendron generation), the size of the guest molecules, and the type of interaction between host and guest molecules (hydrogen bond formation; hydrophobic/hydrophobic interaction), which have been reported in previous literature.[31] However, in this case, the most effective factor is the interaction between host and guest molecule and size of the guest molecule. Maximum loading for 5,7-dibromo-8-hydroxyqunoline, probably, is related to the high polarity of this molecule and the presence of nitrogen heteroatom in this guest molecule, because the presence of nitrogen heteroatom increases the electrostatic interaction between the host and guest molecule. But, minimum loading for ibuprofen is probably related to the low polarity of this molecule and weak interaction between carboxylic acid end functional groups and this guest molecule. Middle position for amount of loaded Congo red is related to the higher polarity but bigger size for this guest molecule relative to the ibuprofen and 5,7-dibromo-8-hydroxyqunoline.



Scheme 1 Linear-dendritic copolymer/guest molecule complexes.

In this case, it seems that other interactions such as hydrophobic/hydrophobic interaction and size of micellar core are not important.

The release of drug from host/guest systems was investigated using usual methods, and products released from linear-dendritic copolymers (as free drugs) were separated according to the method described in the Experimental section, and were detected using UV and IR spectra. The comparison of the data showed that in different pH's the drugs release with different rates.

Here in all cases, the rate of release of guest molecules from linear-dendritic copolymers is faster than

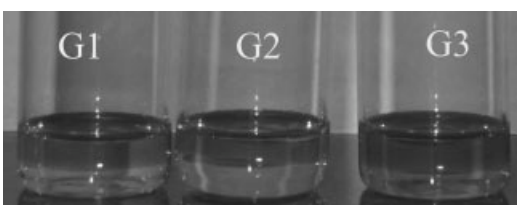


Figure 1 Photograph of chloroform (10 mL) solution of trapped Congo red by linear-dendritic copolymers (0.1 g dendrimer) at 25°C.

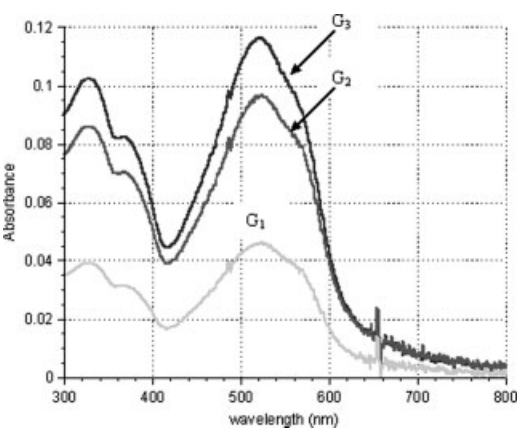


Figure 2 UV spectra of trapped Congo red by linear-dendritic copolymers (0.1 g) in chloroform solution (10 mL) at 25°C.

TABLE I
The Mole Percent of Drugs Trapped by Linear-Dendritic Copolymers at 25°C

Guest molecules	G_1/Drug	G_2/Drug	G_3/Drug
5,7-Dibromo-8-hydroxyqunoline	12	15	23
Ibuprofen	4	9	12
Congo red	6	11	14

release of drug from usual conjugated systems, because here the interaction between host and guest molecules is only physical and there is no chemical bonding between guest molecules and hosts. In the case of Congo red, rate of release is higher than 5,7-dibromo-8-hydoxy quinoline and ibuprofen, because Congo red is a hydrophilic guest molecule and its interaction with aqueous solution is stronger than hydrophobic guest molecules and almost all of guest molecule released after 30 min. Figure 3 shows the release of 5,7-dibromo-8hydroxy quinoline from G_1, G_2, and G_3 in pH 1. Rate of release of drug from nanocarriers with increased generation is increased; this probably is related to steric hindrance of crowded end functional groups in higher generations and weaker interaction between this functional groups and guest molecules. Amount of released drug from G_3 is more than that from G_1 and G_2, and this is logical because transport capacity of G_3 is much more than that of G_2 and G_1. Figure 4 shows the release of 5,7-dibromo-8-hydoxy quinoline from G_1, G_2, and G_3 at pH 10. Here, a logical relation between rate of release and generation cannot be seen. In this pH, the rate of release of drug from G_1 is more than that from G_2 and G_3, the initial rate of release of guest molecule from G_2 is more than that from G_3, but in the end of release this is lower than G_3. It seems that the rate of release and amount of

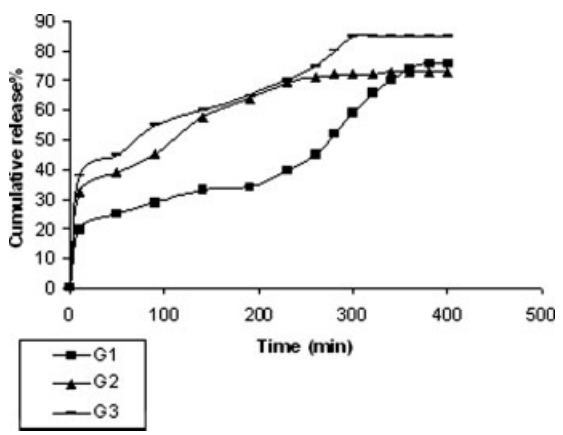


Figure 3 Release of 5,7-dibromo-8-hydoxy quinoline from G_1, G_2, and G_3/5,7-dibromo-8-hydoxy quinoline complexes (70 mg in 5 mL buffer) pH 1 at 37°C.

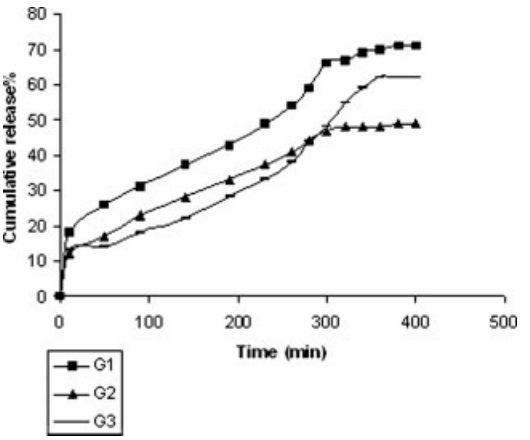


Figure 4 Release of 5,7-dibromo-8-hydoxy quinoline from G_1, G_2, and G_3/5,7-dibromo-8-hydoxy quinoline complexes (70 mg in 5 mL buffer) in pH 10 at 37°C.

released drug depend on several factors, one of them is electrostatic interaction, and there are several other factors (such as conformation and cleavage the ester bond), which may be here are more important than electrostatic interactions. Rate and amount of released drug from guest molecules in pH 7 is slower than other pHs (see Figs. 1–3 in supporting information). Here, rate and amount of release of guest molecule from host molecules increased with increased generation. Figure 5 is related to the release of drug from G_1 in pHs 1, 7, and 10. It can be clearly seen that the highest rate of release of 5,7-dibromo-8-hydroxy quinoline from host molecules is related to the pH 1 and lowest rate of release is related to the pH 7. Interaction between guest molecule and buffer in different pH is important and it seems that interaction between acidic solution and guest molecules containing heteroatoms is more strong than basic or natural solutions which this rise

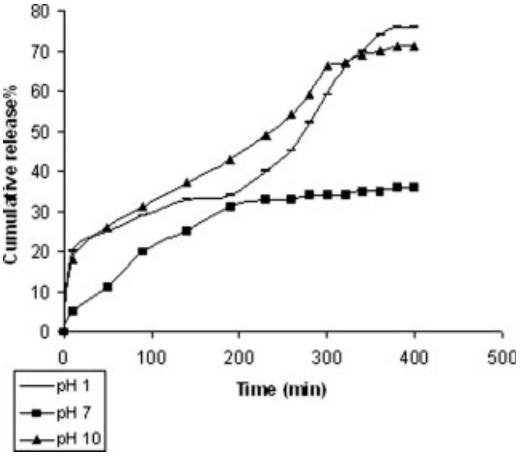


Figure 5 Release of 5,7-dibromo-8-hydoxy quinoline from G_1/5,7-dibromo-8-hydoxy quinoline complex (70 mg in 5 mL buffer) in pH 1, 7 and 10 at 37°C.

to faster release of guest molecule from host molecules at pH 1. Release of 5,7-dibromo-8-hydoxy quinoline and ibuprofen from G_1 and G_2 and G_3 for all conditions can be seen in Figures 4–9 in supporting information.

Release of guest molecules from linear-dendritic host molecules for each guest molecules repeated three times and errors was between 10 and 20%. These almost big errors are related to the physical (weak) interaction between guest and host molecules. Weak interaction between host and guest molecules makes them sensitive to small change in conditions like pH, temperature, and stirring.

CONCLUSIONS

Citric acid and poly(ethylene glycol) are chosen for transportation of some of drugs and model drugs because of their good water solubility, low toxicity, and biocompatibility, which already widely accepted for utilizing in drug formulations. A series of host–guest systems have been developed. The prepared linear-dendritic copolymers/guest molecule complexes are stable at room temperature for a long time. In addition, it was demonstrated that water-soluble linear-dendritic copolymers are able to dissolve polar hydrophobic molecules in aqueous solutions. When guest molecules are drug, the resulting host–guest systems could be considered as the potential drug delivery systems.

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