Polym. Bull. (2012) 68:891–900
DOI 10.1007/s00289-011-0670-1

ORIGINAL PAPER

# Preparation of polyelectrolyte giant capsules using cross-linked alginate gels as core material

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Received: 28 September 2011 / Revised: 11 October 2011 / Accepted: 13 November 2011 /
Published online: 18 November 2011


**Abstract** Polyelectrolyte giant capsules whose diameter is in a millimeter level were prepared by a layer-by-layer deposition of poly(allylamine hydrochloride) (PAH) and poly(styrene sulfonate) (PSS) on an alginate (AGA)-$Ca^{2+}$ sphere followed by dissolution of the AGA core in an ethylenediaminetetraacetic acid or NaCl solution. A fluorescent-labeled bovine serum albumin (BSA) was successfully encapsulated in the PAH/PSS polyelectrolyte capsule. The release of the BSA from the capsule was suppressed in the presence of higher concentration of salt, while the effect of pH was small. In addition, the thickness of the PAH/PSS layer in the capsule wall gave an effect on the release rate of the BSA.

**Keywords** Polyelectrolyte capsule · Layer-by-layer film · Alginate gel · Cross-linked alginate · Protein release

## Introduction

A layer-by-layer (LbL) deposited thin film was first developed by Decher et al. [1–7] and currently widely employed for developing functional thin films. Later, polyelectrolyte microcapsules were prepared by coating the surface of microparticles as core with LbL films followed by dissolution of the core material [8–10]. Various materials such as polymers [11], proteins [12], polysaccharides [13], DNA [14], antibody [15], and nanoparticles [16] have been used to prepare LbL microcapsules. One of the merits of the LbL procedure is that the size of the microcapsules can be controlled in a nano/microscale by choosing an appropriate size of core material. A variety of functional molecules have been successfully

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encapsulated in the cavity of the polyelectrolyte microcapsules. Therefore, polyelectrolyte microcapsules are widely studied for selective transport of ions and molecules [17, 18], biosensors [19–22], and drug delivery devices [23–25].

We report here the preparation of polyelectrolyte giant capsules whose diameter is in a millimeter size. The giant capsules have been prepared using an alginate (AGA)-$Ca^{2+}$ gel as a core material. AGA is an anionic polysaccharide consisting of guluronic acid and mannuronic acid units linked by glycosidic bonds and known to form a gel by cross-linking in the presence of $Ca^{2+}$ ions [26–28]. AGA gels are often used for constructing polyelectrolyte capsules as core because spherical particles can be prepared easily from AGA solution [29]. De Geest and coworkers have recently reported the preparation of giant microcapsules whose diameter was 100–300 μm [30]. However, no report has appeared on the preparation of giant capsule in the millimeter scale. The giant capsules enable us to study the morphological changes during the preparation. It may be interesting to compare the encapsulation and release properties of giant capsules with those of microcapsules reported so far.

## Experimental

### Materials

Sodium AGAs (the viscosity of the 10% aqueous solution is nominally 15–20 cps at 20 °C) were purchased from Funakoshi Co. Ltd. (Tokyo, Japan). Poly(styrenesulfonate) sodium salt (PSS, molecular weight; 500,000) and poly(allylamine hydrochloride) (PAH, molecular weight; 150,000) were from Scientific Polymer Products, Inc. (NY, USA) and Nittobo Co. (Tokyo, Japan), respectively. 4-(*N*,*N*-Dimethylaminosulfonyl)-7-(2-aminoethylamino)-2,1,3-benzoxadiaze (DBD) and fluorescein isothiocyanate (FITC) were obtained from Tokyo Kasei Co. Ltd. (Tokyo, Japan) and Sigma-Aldrich Chemical Co. (Wisconsin, USA), respectively. Other reagents used were of the highest grade available and used without further purification.

DBD-labeled AGA (DBD–AGA) was synthesized as follows. AGA (200 mg) was modified with 5.7 mg of DBD in 100 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid solution (HEPES, pH 7.4) in the presence of *N*-hydroxysuccinimide (2.3 mg) and ethyl(dimethylaminopropyl)carbodiimide (2.8 mg) at 4 °C. The reaction mixture was stirred for 24 h and was purified by dialyzing to distilled water for 3 days and freeze dried. Thus, prepared DBD–AGA contains one DBD residue per ca. 50 monosaccharide units in the AGA chain.

FITC-labeled bovine serum albumin (FITC–BSA) was prepared by reacting BSA and FITC in an aqueous solution at pH 9.0 in usual procedure. The product was purified by dialysis to distilled water for 3 days and lyophilized.

### Apparatus

Fluorescence spectrum was measured using a Shimadzu RF-5300PC spectrofluorophotometer (Kyoto, Japan). Optical microscope images of AGA sphere, polyelectrolyte

film-coated AGA sphere, and polyelectrolyte capsule were recorded on ANMO Dino Lite Pro USB microscope (Taiwan).

## Preparation of AGA sphere, polyelectrolyte film-coated AGA sphere, and polyelectrolyte capsule

AGA sphere, polyelectrolyte film-coated AGA sphere, and polyelectrolyte capsule were prepared as schematically illustrated in Fig. 1. An aqueous AGA solution (2%, with or without 1 mg $mL^{-1}$ protein) was dropped into a 10% $CaCl_2$ solution with a syringe, by which a spherical gel with a diameter in a millimeter level formed through cross-linking AGA chains with $Ca^{2+}$ ions. The resulting AGA sphere was immersed in the $CaCl_2$ solution for 5 min, followed by rinsing in distilled water solution for several seconds two times. Then, the AGA sphere was dispersed in 2 mg $mL^{-1}$ PAH solution containing 1% $CaCl_2$ (pH 7.0) for 15 min to deposit a PAH layer on the surface of the AGA sphere. After rinsing the AGA sphere in 1% $CaCl_2$ solution (pH 7.4) for 1 min, PSS was deposited on the PAH-coated AGA sphere in a similar manner through electrostatic interaction. The deposition of PAH and PSS was repeated to build up 5-bilayer $(PAH/PSS)_5$ film on the surface of the AGA sphere. The $(PAH/PSS)_5$ film-coated AGA sphere was suspended in 100 mM ethylenediaminetetraacetic acid (EDTA) solution for ca. 30 min two times to remove $Ca^{2+}$ ions from the AGA sphere and the sphere was finally immersed in 10 mM HEPES buffer. All experiments were carried out at room temperature ($\sim$20 °C).

## Dissolution of AGA spheres with and without polyelectrolyte film coating

A DBD–AGA sphere was prepared in a similar manner using DBD-labeled AGA to monitor the dissolution with a fluorescence spectrometer. The DBD–AGA sphere without polyelectrolyte coating was placed in 10 mL of EDTA or NaCl solution (10 mM HEPES buffer, pH 7.4) and the solution was gently stirred. The fluorescence intensity of the solution was measured at 579 nm every 10 min (ex: 425 nm) until the sphere was completely dissolved. The $(PAH/PSS)_5$ film-coated sphere was similarly prepared and dissolution of DBD–AGA gel in the sphere was

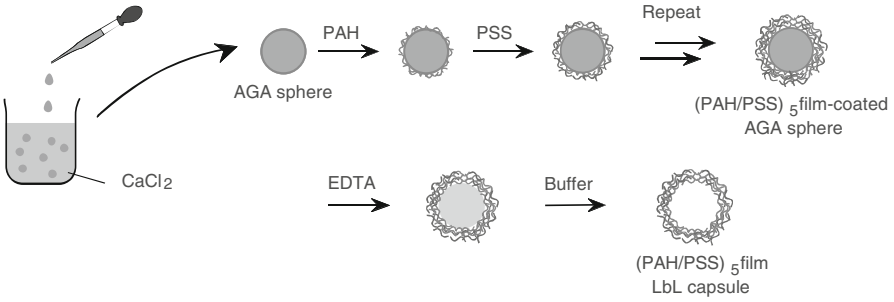


**Fig. 1** Preparation of AGA sphere, polyelectrolyte film-covered AGA sphere, and polyelectrolyte capsule

monitored in a similar manner. At the end of monitoring, the resulting $(PAH/PSS)_5$ polyelectrolyte capsule was collapsed with a pair of tweezers to record the fluorescence intensity corresponding to 100% release of DBD–AGA.

### Encapsulation of FITC–BSA in the polyelectrolyte capsules and release

AGA sphere was prepared using AGA solution containing FITC–BSA (1 mg $mL^{-1}$) and the surface of the sphere was coated with $(PAH/PSS)_5$ film. For the preparation of FITC–BSA-containing $(PAH/PSS)_5$ capsules, the AGA core was dissolved in EDTA solution and treated in 10 mM HEPES buffer. After rinsing in water, one piece of the sphere was put in a quartz cuvette (10 × 10 $mm^2$) which was filled with 3 mL solution to monitor the release of FITC–BSA. The intensity of fluorescence arising from FITC moiety was occasionally recorded at 520 nm (ex: 494 nm). During the measurement, the AGA sphere and capsule did not contribute to the fluorescence intensity of the supernatant because they were located on the bottom of the cuvette.

## Results and discussion

### Preparation of AGA sphere and its dissolution

An aliquot of aqueous DBD-labeled AGA solution was dropped into a 10% $CaCl_2$ solution, affording DBD–AGA spheres with a diameter in a millimeter size. Before preparation of polyelectrolyte capsules, the dissolution behavior of the AGA spheres in EDTA and NaCl solutions was studied. Figure 2 shows dissolution kinetics of the DBD–AGA sphere in different concentration of EDTA solutions. The DBD–AGA sphere was completely dissolved in the EDTA solutions in 20–100 min, depending on the concentration of EDTA. It is well known that AGA gels cross-linked with $Ca^{2+}$ ions can be dissolved in the presence of EDTA [29]. On the contrary, virtually

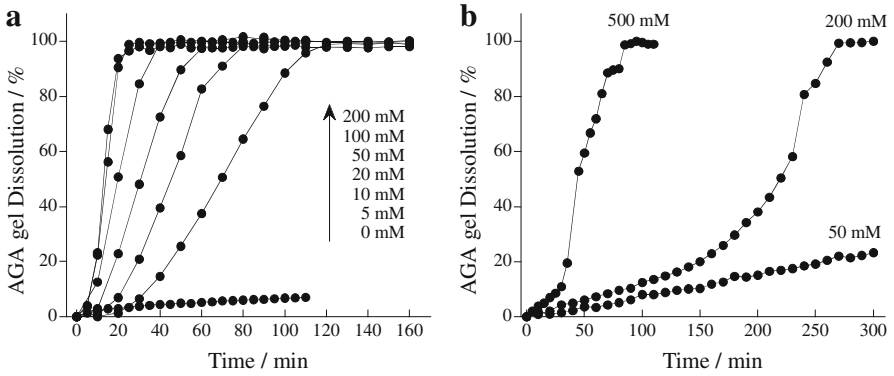


**Fig. 2** Dissolution of DBD–AGA sphere in 10 mL of 10 mM HEPES buffer solutions (pH 7.4) containing EDTA (**a**) and NaCl (**b**)

no dissolution was observed in the EDTA-free HEPES buffer. It has been reported that $Ca^{2+}$ ion-cross-linked AGA microgels are unstable in the presence of high concentration of NaCl due to the replacement of $Ca^{2+}$ ions with $Na^{+}$ ions [26]. Figure 2 shows the stability of the DBD–AGA sphere in NaCl solutions. The DBD–AGA sphere was completely dissolved in 500 mM NaCl solution within 100 min, while the full dissolution took ca. 300 min in 200 mM NaCl solution. On the other hand, the dissolution of the DBD–AGA sphere was slow in 50 mM NaCl solution. These results show that the DBD–AGA spheres can be dissolved in the presence of NaCl as well as in EDTA solution. Consequently, cross-linked AGA spheres should not be handled in the solutions containing high concentration of NaCl. For the preparation of polyelectrolyte capsules using AGA spheres as core, use of EDTA solution is recommended for dissolving the AGA core.

The size and shape of the AGA sphere before and after coating with LbL polyelectrolyte films was evaluated using optical microscope. Figure 3 shows microscope images of uncoated AGA sphere (Fig. 3a), $(PAH/PSS)_5$ film-coated AGA sphere before (Fig. 3b) and after EDTA processing (Fig. 3c), and after successive treatment in 100 mM HEPES buffer (Fig. 3d). The AGA sphere used here exhibited a smooth surface and the diameter was ca. 2.6 mm (Fig. 3a). After coating the $(PAH/PSS)_5$ film on the AGA sphere, the diameter of the coated sphere was slightly increased to 2.8 mm (Fig. 3b) probably due to uptake of water during the deposition of the $(PAH/PSS)_5$ film. On the other hand, the $(PAH/PSS)_5$ film-coated AGA sphere was significantly shrunken after being immersed in 100 mM EDTA solution for 60 min (Fig. 3c). The shrunken sphere was expanded to the original size upon being immersed in 100 mM HEPES buffer at pH 7.4 (Fig. 3d). Figure 3e, f shows a polyelectrolyte capsule broken by pinching with tweezers, which suggests that the capsule contains no AGA gel after processing in the HEPES buffer.

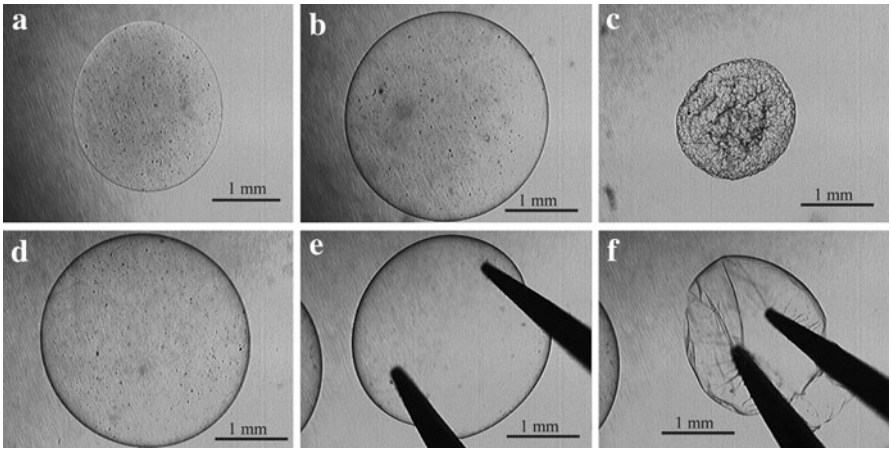


**Fig. 3** Optical microscope images of AGA sphere (**a**), $(PAH/PSS)_5$ film-coated AGA sphere before (**b**) and after immersed in 100 mM EDTA solution for 5 min (**c**), and $(PAH/PSS)_5$ film-coated AGA sphere after successive immersion in 100 mM HEPES buffer for 3 min (**d**). **e** and **f** show a capsule broken

The kinetics of release of DBD–AGA from the sphere was studied for elucidating the factors which determine the morphological changes shown in Fig. 4. Figure 4 plots a cumulative amount of DBD–AGA released from the $(PAH/PSS)_5$ film-coated DBD–AGA sphere into 200 mM EDTA and 500 mM NaCl solutions as a function of time. When the $(PAH/PSS)_5$ film-coated sphere was immersed in the 200 mM EDTA solution, virtually no DBD–AGA was released from the sphere for 200 min though the sphere was shrunken as was shown in Fig. 3c. These results suggest that water was squeezed out of the sphere by osmotic pressure. It is most likely that DBD–AGA gel dissolved in the sphere because of a chelation of $Ca^{2+}$ ions by EDTA and the resulting free water diffused out of the sphere across the $(PAH/PSS)_5$ film because the ionic concentration of the EDTA solution was higher than that in the sphere. On the other hand, DBD–AGA was unable to permeate the $(PAH/PSS)_5$ film probably due to a decreased size of the pores in the film as a result of the shrinkage. The shrunken sphere restored its original size within a few min upon replacing the EDTA solution with 10 mM HEPES buffer and DBD–AGA was rapidly released out of the sphere. A different behavior was observed when the $(PAH/PSS)_5$ film-coated sphere was immersed in 500 mM NaCl solution. DBD–AGA was released slowly in the NaCl solution as a result of dissolution of the DBD–AGA gel by NaCl. No shrinkage of the sphere was observed in the NaCl solution (images not shown).

## Release of protein from $(PAH/PSS)_5$ film capsule

It may be possible to encapsulate proteins in polyelectrolyte film-coated AGA spheres and microcapsules because cross-linked AGA gels can entrap proteins [26–29]. Therefore, it is interesting to evaluate the encapsulation of protein in the $(PAH/PSS)_5$ film-coated giant capsules and its release properties.

The encapsulation of FITC–BSA and its release from AGA sphere, $(PAH/PSS)_5$ film-coated AGA sphere, and $(PAH/PSS)_5$ giant capsule was studied. It was possible to encapsulate FITC–BSA in the AGA sphere by dropping a mixed solution of FITC–BSA and AGA into 10% $CaCl_2$ solution, by which $(PAH/PSS)_5$ film-coated

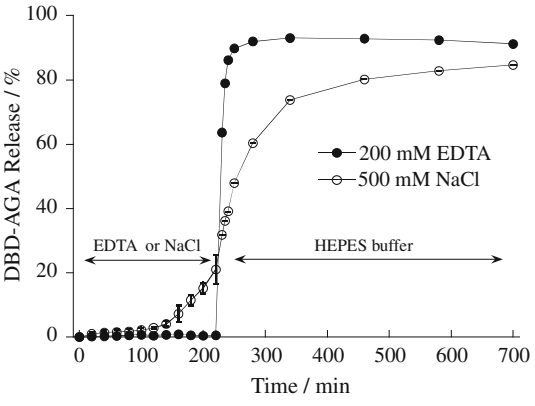


**Fig. 4** Release of DBD–AGA from the $(PAH/PSS)_5$ film-coated DBD–AGA sphere in EDTA and NaCl solutions. The $(PAH/PSS)_5$ film-coated DBD–AGA sphere was immersed in 500 mM NaCl (*open circle*) and 200 mM EDTA solutions (*closed circle*) for the first 200 min and the solution was replaced with 10 mM HEPES buffer (pH 7.4)

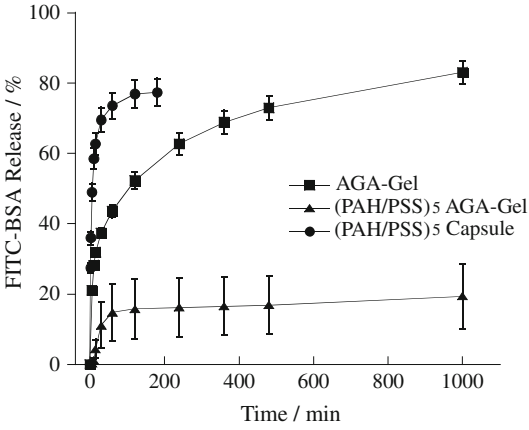


**Fig. 5** Release of FITC–BSA from uncoated AGA sphere, $(PAH/PSS)_5$ film-coated AGA sphere, and $(PAH/PSS)_5$ giant capsule in 10 mM HEPES buffer (pH 7.4)

AGA spheres and $(PAH/PSS)_5$ giant capsules were also prepared. Figure 5 shows release kinetics of FITC–BSA from the spheres and capsule. FITC–BSA rapidly released from the capsule while the release was suppressed for the $(PAH/PSS)_5$ film-coated and uncoated AGA spheres. It is clear that the diffusion of FITC–BSA across the $(PAH/PSS)_5$ film is the rate-limiting step for the release from the capsule. In contrast, for the release from the spheres, the rate-limiting step may be the diffusion of FITC–BSA in the AGA gel. The slow release of FITC–BSA from the uncoated AGA sphere suggests a slow diffusion of FITC–BSA in the AGA gel due to a high molecular weight of FITC–BSA (Mw; ca. 66,000). The release of FITC–BSA from the $(PAH/PSS)_5$ film-coated AGA sphere was very slow and only ca. 19% was released after 1,000 min. These results suggest that the $(PAH/PSS)_5$ film provides further barrier to the release of FITC–BSA.

Next, we have evaluated the release properties of FITC–BSA from the polyelectrolyte capsule under different conditions. Figure 6 shows the effects of pH, ionic strength, and the thickness of the (PAH/PSS) film on the release rate. Figure 6a shows that the effect of pH on the release profile is negligibly small in the pH range tested. In this context, we have separately found that the size of the capsule remains unchanged in the range of pH 6.0–9.0, suggesting that the permeability of the capsule's wall was unchanged. Matsuyama and coworkers have recently reported that the size of (PAH/PSS) film-coated microgel remained unchanged in pH 5.0–9.0 [31]. It is probable that virtually no structural change occurred in the $(PAH/PSS)_5$ film in the pH range. On the other hand, the release of FITC–BSA was suppressed in the presence of 400 mM NaCl (Fig. 6b). The permeability of LbL films is often enhanced by increased ionic strength [32]. However, in some cases, the permeability of PSS-containing LbL film can be suppressed in the presence of high concentration of salts ion [33]. Figure 6c shows the release profile of FITC–BSA from the capsules consisting of $(PAH/PSS)_n$ ($n = 5$, 10, 15, and 20) films. The release rate became lower with increasing the film thickness, as expected, though the effect was not so significant.

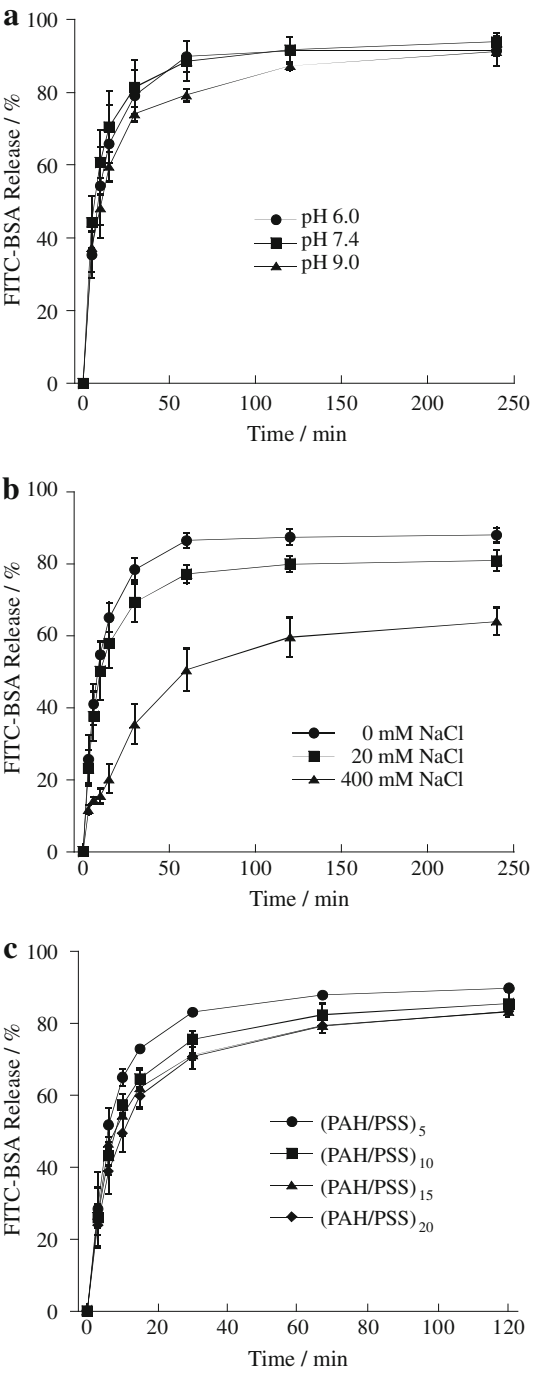


**Fig. 6** The release kinetics of FITC–BSA from $(PAH/PSS)_5$ capsule at different pH (**a**) and ionic strength (**b**) and the effects of film thickness (**c**). The medium used was 10 mM HEPES buffer at pH 7.4 unless otherwise stated

## Conclusions

Polyelectrolyte giant capsules were successfully prepared by LbL deposition of PAH and PSS on an AGA gel sphere as core followed by dissolution of the core in EDTA and HEPES solutions. FITC–BSA can be encapsulated in the AGA sphere, $(PAH/PSS)_5$ film-coated AGA sphere, and $(PAH/PSS)_5$ film capsule. The release of FITC–BSA from the capsule was accelerated as compared to the relatively slow release from the uncoated and $(PAH/PSS)_5$ film-coated AGA spheres. The release rate of FITC–BSA from the capsule can be tuned to some extent by controlling ionic strength and the thickness of the capsule wall. The polyelectrolyte giant capsules may be used as containers or reactors.

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