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Short Communication

Voltammetric Bahavior of Ferricyanide Ion-confined LbL Film-coated Electrodes: Use of Heparin and κ-Carrageenan as Film Component

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Heparin (HEP) and κ-carrageenan (CAR) were used for constructing thin films on the surface of a gold (Au) electrode and redox properties of ferricyanide ions, $Fe(CN)_6^{3-}$, on the film-coated electrodes were studied. HEP- and CAR-containing thin films were successfully prepared by layer-by-layer (LbL) deposition of the polysaccharides and poly(allylamine hydrochloride) (PAH). $Fe(CN)_6^{3-}$ ions penetrated the LbL-deposited films to exhibit redox reactions on the Au electrodes when the outermost surface of the film was covered with PAH. On the other hand, penetration of $Fe(CN)_6^{3-}$ ions was suppressed when the surface of the film was covered with HEP or CAR. The results were rationalized on the basis of electrostatic repulsion between $Fe(CN)_6^{3-}$ anion and negative charges in HEP and CAR. In addition, $Fe(CN)_6^{3-}$ ions were confined in the LbL films and the confined $Fe(CN)_6^{3-}$ ions were successfully used for the electrocatalytic oxidation of ascorbic acid.

Keywords: layer-by-layer film, polysaccharide, ferricyanide ion, heparin, carrageenan

1. INTRODUCTION

Layer-by-layer (LbL) film-modified electrodes have been widely studied for the development of sensors [1-5], fuel cells [6], and controlled release [7-10]. LbL films are prepared by an alternate deposition of polymeric materials on solid surfaces through electrostatic [11,12], hydrogen bonding [13, 14], and biological interactions [15, 16]. Polymeric materials used so far include synthetic polymers [17-19] as well as biopolymers such as proteins [20, 21], polysaccharides [22, 23], and DNA

[24]. Among biopolymers, polysaccharides have attracted much attention as building block of LbL films because of their swelling ability and biocompatibility [25].

Recently, we have reported that a gold (Au) electrode can successfully be coated with LbL films consisting of carboxymethylcellulose (CMC) and alginic acid (ALG) [26, 27]. Interestingly, $Fe(CN)_6^{3-}$ ions were confined in the polysaccharide LbL films to exhibit redox reactions on the Au electrode. It was shown that the $Fe(CN)_6^{3-}$ ions are confined through electrostatic bonding to the positive sites in the LbL films originating from poly(amine)s used as counter polymer. Thus, the $Fe(CN)_6^{3-}$ ions were able to mediate electron transfer from ascorbic acid in solution to the Au electrode [28]. In this regard, it is interesting to use different type of polysaccharides other than CMC and ALG as component of LbL films because physical and chemical properties of LbL films often significantly depend on the type of materials. For this reason, in the present study, we have employed sulfonated polysaccharides, *i.e.*, heparin (HEP) and κ-carrageenan (CAR), in contrast to CMC and ALG which contain carboxylate groups. In fact, we have found that $Fe(CN)_6^{3-}$ ions are confined also in the HEP and CAR LbL films and the LbL film-coated electrodes can be used for the electrocatalytic oxidation of ascorbic acid.

2. EXPERIMENTAL

2.1 Reagents

Heparin (HEP) and κ-carrageenan (CAR) were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA). Poly(allylamine hydrochloride) (PAH, average molecular weight (MW): 70,000) and poly(ethyleneimine) (PEI, MW: 60,000-80,000) were purchased from Nittobo Co. (Osaka, Japan) and Nakalai Tesque Co (Kyoto, Japan), respectively. The chemical structures of HEP and CAR are illustrated in Fig. 1. Sodium 3-mercapto-1-propanesulfonate (MPS) was purchased from Tokyo Kasei Co. (Tokyo, Japan). All other reagents used were of the highest grade available and were used without further purification.

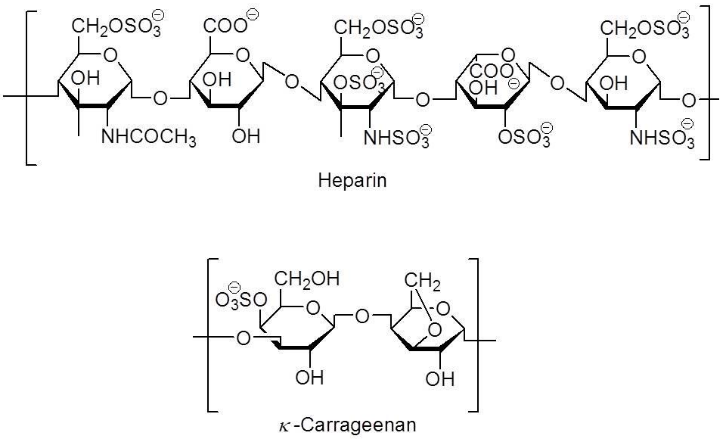

Figure 1. Chemical structures of heparin and κ-carrageenan.

2.2 Apparatus

A quartz-crystal microbalance (QCA 917, Seiko EG & G, Tokyo, Japan) was used for the gravimetric analysis of LbL films. A 9-MHz AT-cut quartz resonator coated with a thin Au layer (surface area, 0.2 cm^2) was used as a probe, in which adsorption of 1 ng of substance induces a -0.91 Hz change in the resonance frequency. All electrochemical measurements were carried out using an electrochemical analyzer (ALS, model 660B).

2.3 Preparation of electrodes

LbL films were prepared on the surface of Au disk electrode (3-mm diameter), according to the reported procedure [26, 27]. The surface of the Au electrode was polished thoroughly using alumina slurry and rinsed in distilled water before use. The polished Au electrode was cleaned in a 0.5 M H_2SO_4 solution by scanning the electrode potential from -0.2 to 1.5 V vs. Ag/AgCl at 0.1 Vs^{-1} for 15 min. The surface of the Au electrode was first modified with MPS and PEI by immersing the electrode in 1 mg mL^{-1} aqueous solution of MPS overnight and rinsed, followed by the treatment in a 1 mg mL^{-1} aqueous solution of PEI for 30 min. The PEI-modified electrode was then coated with LbL film by dipping it alternately in a 0.5 mg mL^{-1} HEP or CAR solution (10 mM Tris-HCl buffer containing 150 mM NaCl, pH 7.0) and a 0.5 mg mL^{-1} PAH solution (10 mM Tris-HCl buffer containing 150 mM NaCl, pH 7.0) for 30 min with an intermediate 10-min rinse in the working buffer. The above procedure was repeated for depositing desired number of layers of LbL film on the electrode.

2.4 Quartz crystal microbalance (QCM)

The surface of Au-coated quartz resonator was electrochemically cleaned in a 0.5 M H_2SO_4 solution before use. The quartz resonator was first modified with MPS and PEI. Then, LbL films were deposited on both surfaces of the quartz resonator in a similar manner to the film deposition on Au electrode. The film-deposited probe was rinsed in pure water for 1 min and dried in air after each deposition until the resonance frequency showed a steady-state value to estimate the weight of the film.

2.5 Confinement of $Fe(CN)_6^{3-}$ ion in the LbL films

$Fe(CN)_6^{3-}$ ion was confined in the LbL films by immersing the LbL fim-coated electrode in a 1 mM $K_3Fe(CN)_6$ solution (10 mM Tris-HCl buffer containing 150 mM NaCl, pH 7.0) for 15 min after each deposition of the HEP-PAH or CAR-PAH bilayer. The electrochemical response of the electrodes was measured in a glass cell using the LbL film-modified electrode as working electrode, a platinum wire as counter electrode, and a Ag/AgCl electrode (3.3 M KCl) as reference electrode. All measurements were performed in 10 mM Tris-HCl buffer containing 150 mM NaCl (pH 7.0) under air at room temperature (~ 20 °C).

3. RESULTS AND DISCUSSION

3.1 Characterization of LbL films by QCM

HEP-PAH and CAR-PAH films were deposited on the surface of a quartz resonator to evaluate the deposition behavior of the films. The change in resonance frequency (ΔF) was recorded after drying in air to exclude possible effects of bound water on the ΔF values. Fig. 2 plots the -ΔF values as a function of the number of bilayers. For both films, the -ΔF values increased with the increasing number of layers in the films, showing the LbL films were successfully deposited on the surface of the quartz resonator. It is likely that the film formation relies on the electrostatic binding between the sulfonate residues in HEP and CAR and positively charged PAH chains. The thickness of the films in the dry state is estimated to be ~32 nm for the PEI(HEP-PAH)$_5$ film and ~50 nm for the PEI(CAR-PAH)$_5$ film from the QCM results, assuming that the density of the LbL films is ~1.2 g cm^{-3} [29].

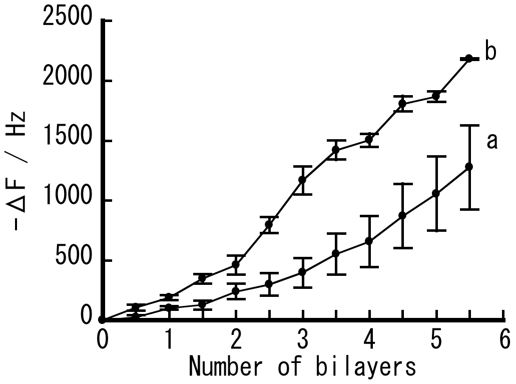

Figure 2. Frequency changes in the QCM for the deposition of HEP-PAH (a) and CAR-PAH films (b) on the PEI-coated quartz resonator. The integers in the abscissa represent the deposition of PAH. The average values of three independent measurements are plotted.

3.2 Cyclic voltammetry of LbL film-coated electrodes

Cyclic voltammetry was employed for evaluating redox properties of Fe(CN)$_6^{3-}$ ions on the LbL film-modified electrodes. Figs. 3a and 3b show cyclic voltammograms (CVs) of Fe(CN)$_6^{3-}$ ions on the Au electrodes coated with (HEP-PAH)$_n$ and (CAR-PAH)$_n$ films, respectively, where n denotes the number of bilayers in the LbL films. The CVs were recorded in a 1 mM K$_3$Fe(CN)$_6$ solution at pH 7.0 after the electrode had been immersed in the solution for 15 min. For the electrode coated with thinner HEP-PAH film, the CV showed a well-defined redox couple at 0.2-0.3 V accompanied by a weak signals at the higher potential region (n=1 in Fig 3a). The separation between the anodic and cathodic peak potentials (ΔE$_p$) for the redox couple at 0.2-0.3 V was ~60 mV, suggesting a diffusion-controlled reversible one-electron redox reactions of Fe(CN)$_6^{3-}$ ions occurred. On the other hand, the shape and size of the CVs changed on the thicker HEP-PAH film-coated electrodes. A pair of new redox peaks appeared at around 0.35 V in the CVs recorded on the (HEP-PAH)$_n$ film-coated electrodes (n=2~5 in Fig. 3a). The intensity of the redox peaks increased with the increasing number of layer in the LbL film, suggesting a different mechanism in the redox reaction of $Fe(CN)_6^{3-}$ ions is involved in the thicker film-coated electrodes. A similar trend was observed also in the CVs recorded on the $(CAR\text{-}PAH)_n$ film-coated electrodes (Fig. 3b). Presumably, $Fe(CN)_6^{3-}$ ions were confined in the thicker $(HEP\text{-}PAH)_n$ and $(CAR\text{-}PAH)_n$ films judging from the CVs with small ΔE_p values for the redox couples in the higher potential range.

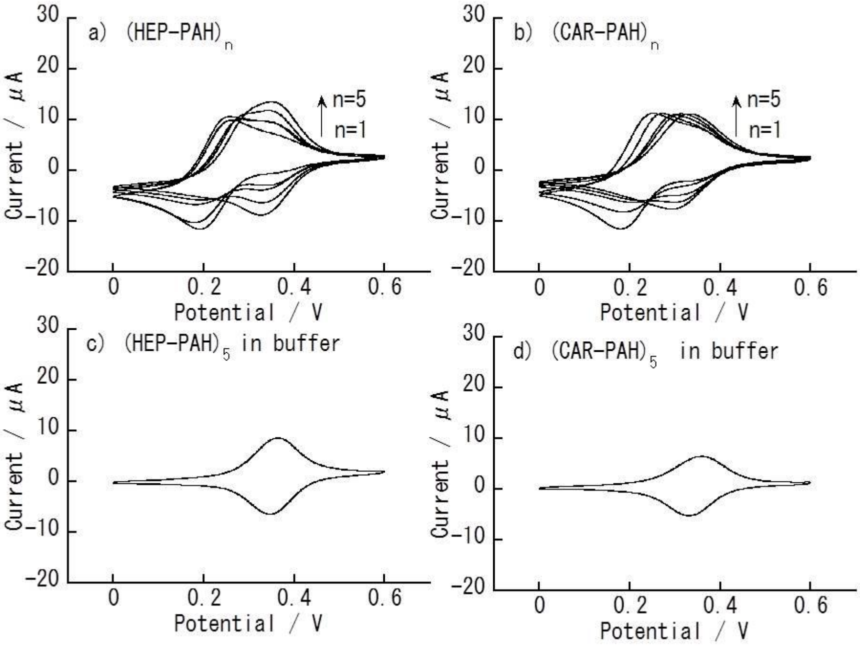

Figure 3. (a and b) CVs of 1 mM $K_3Fe(CN)_6^{3-}$ on Au electrodes coated with the $(HEP\text{-}PAH)_n$ (a) and $(CAR\text{-}PAH)_n$ films (b) as a function of the number of bilayers in the films. (c and d) CVs of $Fe(CN)_6^{3-}$ ions confined in the $(HEP\text{-}PAH)_5$ (c) and $(CAR\text{-}PAH)_5$ films (d) on the Au electrode. The CVs were recorded in a 10 mM Tris-HCl buffer containing 150 mM NaCl at pH 7.0 in the presence (a and b) and absence of 1 mM $K_3Fe(CN)_6$ (c and d). Scan rate was 50 mV s^{-1}.

To confirm the confinement of $Fe(CN)_6^{3-}$ ions in the LbL films, the $(HEP\text{-}PAH)_5$ and $(CAR\text{-}PAH)_5$ film-coated electrodes were rinsed in the working buffer for 15 min after the CVs had been recorded in the $K_3Fe(CN)_6$ solution. Then, CVs of the electrodes were recorded in the buffer solution without $Fe(CN)_6^{3-}$ ion (Figs. 3c and 3d). The LbL film-coated electrodes exhibited clear redox waves even in the absence of $Fe(CN)_6^{3-}$ ions in solution. The ΔE_p values for the $(HEP\text{-}PAH)_5$ and $(CAR\text{-}PAH)_5$ film-coated electrodes were ~20 and 30 mV, respectively. These results clearly show that the $(HEP\text{-}PAH)_5$ and $(CAR\text{-}PAH)_5$ film-coated electrodes contain a significant amount of $Fe(CN)_6^{3-}$ ions in the LbL films. Thus, the sulfonic acid-bearing polysaccharides are useful for immobilizing $Fe(CN)_6^{3-}$ ions on the electrode.

It is interesting to evaluate the effects of electric charges on the outermost surface of LbL film on the redox reaction of $Fe(CN)_6^{3-}$ ions. It has been reported that $Fe(CN)_6^{3-}$ ions barely penetrate the LbL films composed of synthetic polymers such as poly(styrene sulfonate) and poly(acrylic acid) due to the electrostatic repulsion when the outermost surface is covered with these polyanions [30, 31].

Therefore, we have prepared HEP- and CAR-terminated LbL film-coated electrodes and studied the redox properties of $Fe(CN)_6^{3-}$ ions. Fig. 4 shows CVs of $Fe(CN)_6^{3-}$ ions on the Au electrodes coated with $(HEP\text{-}PAH)_nHEP$ and $(CAR\text{-}PAH)_nCAR$ films (Figs. 4a and 4b) and CVs recorded in the $Fe(CN)_6^{3-}$ ion-free solution (Figs. 4c and 4d). The redox reaction of $Fe(CN)_6^{3-}$ ions was severely suppressed on the $(HEP\text{-}PAH)_nHEP$ film-coated electrodes and very low response was observed in the potential range of 0.2-0.4 V (Fig. 4a).

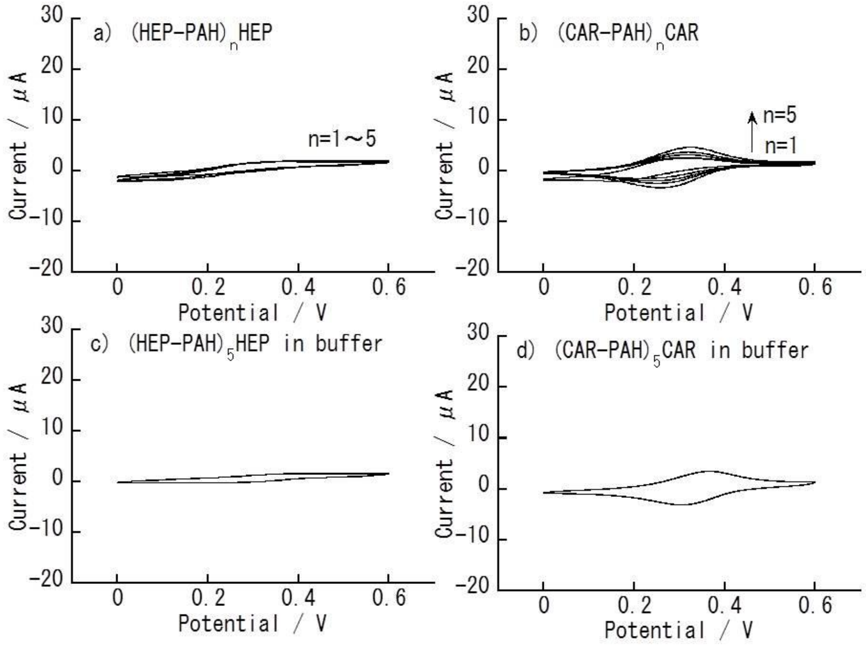

Figure 4. (a and b) CVs of 1 mM $K_3Fe(CN)_6^{3-}$ on Au electrodes coated with the $(HEP\text{-}PAH)_nHEP$ (a) and $(CAR\text{-}PAH)_nCAR$ films (b) as a function of the number of bilayers in the films. (c and d) CVs of $Fe(CN)_6^{3-}$ ions confined in the $(HEP\text{-}PAH)_5HEP$ (c) and $(CAR\text{-}PAH)_5CAR$ films (d) on the Au electrode. The CVs were recorded in a 10 mM Tris-HCl buffer containing 150 mM NaCl at pH 7.0 in the presence (a and b) and absence of 1 mM $K_3Fe(CN)_6$ (c and d). The scan rate was 50 mV s^{-1}.

These results show that $Fe(CN)_6^{3-}$ ions did not penetrate the $(HEP\text{-}PAH)_nHEP$ films probably due to the electrostatic repulsion between the negative charges on the film surface and the $Fe(CN)_6^{3-}$ ion. On the other hand, weak redox peaks were observed in the CVs recorded on the $(CAR\text{-}PAH)_nCAR$ film-coated electrodes, suggesting $Fe(CN)_6^{3-}$ ions penetrated the LbL films to some extent. The different behavior in CVs on the $(CAR\text{-}PAH)_nCAR$ film-coated electrodes from that on the $(HEP\text{-}PAH)_nHEP$ film-coated electrodes may originate from the different charge density of HEP and CAR. As illustrated in Fig. 1, CAR contains one negative charge in every two saccharide residues while every saccharide unit in HEP contains one or two negative charges. Thus, the charge density on the CAR chains is much lower than that on the HEP chains. Consequently, $Fe(CN)_6^{3-}$ ions are nearly completely excluded from the LbL films covered with highly negatively-charged HEP. This is a clear contrast to the reported results for the LbL films composed of carboxylate-bearing polysaccharides such as ALG and CMC, in which $Fe(CN)_6^{3-}$ ions were penetrable to some extent even if the outermost surface was covered with the negatively-charged polysaccharides [26, 27].

The stability of the $Fe(CN)_6^{3-}$ ions confined in the $(HEP\text{-}PAH)_5$ and $(CAR\text{-}PAH)_5$ films was evaluated by monitoring the CV response. The $Fe(CN)_6^{3-}$ ion-confined electrodes were stored in 10 mM Tris-HCl buffer containing 150 mM NaCl (pH 7.0) and CV was recorded occasionally in the working buffer (the buffer solution was replaced with a new one upon CV measurements). For both electrodes, the peak current in the CV was significantly decreased in the first 30 min, suggesting that some of the $Fe(CN)_6^{3-}$ ions were released rapidly out of the LbL films and, after 30 min, 50-60% of the $Fe(CN)_6^{3-}$ ions remained in the films. The percentage of $Fe(CN)_6^{3-}$ ions retained in the LbL films was 40-50% after 3-h storage. Thereafter, the release rates were relatively slow although the release continued.

3.3 Electrocatalysis on the LbL film-coated electrodes

Electrocatalytic oxidation of ascorbic acid on the $Fe(CN)_6^{3-}$ ion-confined electrodes was studied. $Fe(CN)_6^{3-}$ ion has been widely used for determining ascorbic acid [32,33].

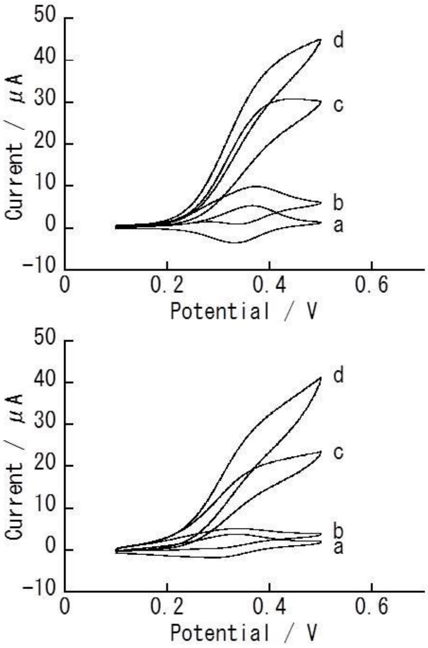

Figure 5. CVs of the $(HEP\text{-}PAH)_5$ film- (top) and $(CAR\text{-}PAH)_5$ film-coated Au electrodes (bottom) in the absence and presence of ascorbic acid. The LbL films were impregnated with $Fe(CN)_6^{3-}$ ions before use. Sample solutions contain 0 mM (a), 1 mM (b), 5 mM (c), and 10 mM (d) ascorbic acid in 10 mM Tris-HCl buffer containing 150 mM NaCl at pH 7.0. The scan rate was 50 mV s^{-1}.

Fig. 5 shows CVs of the $Fe(CN)_6^{3-}$ ion-confined film-coated electrodes in the absence and presence of ascorbic acid. The oxidation current at 0.3-0.4 V originating from the $Fe(CN)_6^{3-}$ ions increased in the presence of ascorbic acid depending on the concentration. These results suggest that ascorbic acid is catalytically oxidized by the $Fe(CN)_6^{3-}$ ions in the film because no oxidation peak can be observed for ascorbic acid in this potential range on the LbL film-coated electrodes without $Fe(CN)_6^{3-}$ ion. It has been reported that ascorbic acid is catalytically oxidized by $Fe(CN)_6^{3-}$ ion according to equations (1) and (2) [28]. The confined $Fe(CN)_6^{3-}$ ions oxidized ascorbic acid according to the equation (1) to generate the reduced form, $Fe(CN)_6^{4-}$, which was electrochemically re-oxidized into $Fe(CN)_6^{3-}$ ions (Eq. 2). The results show that the $Fe(CN)_6^{3-}$ ion-confined electrodes are useful for the electrocatalytic oxidation of ascorbic acid in solution.

$$\text{ascorbic acid} + 2Fe(CN)_6^{3-} \rightarrow \text{dehydroascorbic acid} + 2Fe(CN)_6^{4-} \quad (1)$$

$$Fe(CN)_6^{4-} \rightarrow Fe(CN)_6^{3-} + e- \quad (2)$$

4. CONCLUSION

HEP and CAR were successfully used for constructing LbL film coatings on Au electrode. $Fe(CN)_6^{3-}$ ions were confined in the LbL films when the outermost surface of the LbL film was covered with polycation or CAR. In contrast, $Fe(CN)_6^{3-}$ ions did not penetrate the LbL films terminated with HEP due to an electrostatic repulsion between the negative charges in HEP and $Fe(CN)_6^{3-}$ anion. The $Fe(CN)_6^{3-}$ ion-confined electrodes were used for the electrocatalytic oxidation of ascorbic acid in solution. The HEP- and CAR-based LbL films may be useful for the development of electrocatalytic systems in biosensors and bioreactors.

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