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Membrane dynamics in the intact PM2 phage and its host cells as monitored by $T_{1\rho}$(H)

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Abstract

Temperature dependence of the spin-lattice relaxation time of proton in the rotating frame ($T_{1\rho}$(H)) was examined for the membranes of the intact PM2 phage, its host bacterial cells, and the phospholipids extracted from the cells. The relevant motions of the phospholipid molecules in all lipid membranes were found in the fast-motional regime ($\tau_c < 1.7 \times 10^{-6}$ s) in the temperature range from 0 to 34°C. The motions responsible for the relaxation in the intact biomembranes are more suppressed than those of the extracted phospholipid bilayers, suggesting that the lipid–protein interactions induce slow motions of the phospholipids in the membrane. Especially, the membrane of the intact PM2 phage showed a cooperative change in the motional state, being consistent with the reported change in the phosphorus chemical shift anisotropies of DNA and phospholipids of the phage particle. © 1998 Elsevier Science B.V.

Keywords: Solid-state NMR; $T_{1\rho}$; In vivo NMR; Virus

1. Introduction

Nuclear magnetic resonance (NMR) is now widely used for the investigations of small and soluble metabolic molecules in many intact biological systems [1]. Supramolecular structures such as biomembranes also play important roles in the biological processes. However, they are not yet widely investigated in vivo, since it is not easy to work on the supramolecular structures in intact biological systems. Even for such a simple system as human erythrocytes, a sophisticated labeling method is needed to investigate the membrane [2]. In the previous works [3–6], we have successfully shown that ^{1}H–^{31}P cross-polarization technique is a powerful method for the study of supramolecular structures such as biomembranes and huge nucleic acids in intact biological systems. Since the intact systems usually carry a lot of phosphorus-containing molecules, the specific information on each component should be separately obtained. We have selectively observed the ^{31}P-NMR spectra of the biomembranes of a marine bacterium, *Alteromonas espejiana*, and its bacteriophage PM2 by the use of ^{1}H–^{31}P cross-polarization method [3]. PM2 is a

Abbreviations: $T_{1\rho}$(H), the spin-lattice relaxation time of proton in the rotating frame; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; Cell PL, the total phospholipid extracted from *A. espejiana*; PM2 PL, a mixture of PG and PE at the ratio found in PM2 phage (semi PM2 total phospholipid)

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spherical bacteriophage containing lipid. The phase behaviors of the membranes were discussed on the basis of the phosphorus chemical shift an-isotropy estimated from the membrane spectrum [3]. The phosphorus chemical shift anisotropy represents the bulk property of the polar head groups of the membrane.

The cross polarization method can also provide us with the information on the spin-lattice relaxation time of proton in the rotating frame ($T_{1\rho}$(H)). $T_{1\rho}$(H) is a function of the correlation time and second moment of motions [7]. In contrast to the chemical shift anisotropy, $T_{1\rho}$(H) is dominated by the motion of the lipid molecules most efficient for the relaxation mechanism. It is sensitive to the slow motions which reflect the phase behavior of lipid bilayers [8] and their interactions with other molecules [9]. This work is devoted to the exploration of the dynamic information of biomembranes in the intact biological systems, namely, the PM2 phage and its host cell in terms of the spin-lattice relaxation time of proton in the rotating frame ($T_{1\rho}$(H)).

2. Materials and methods

Purification and preparation of the bacteriophage PM2 and the host cells (*Alteromonas espejiana*) have been described previously [3,4]. Phospholipids were extracted according to the method of Bligh and Dyer [10], and further purified by silicic acid column chromatography. The semi PM2 total phospholipid is a mixture of phophatidylethanolamine (PE) and phosphatidylglycerol (PG) obtained from the host cells at the ratio found in the PM2 phage (PE/PG = 23/64). For NMR measurements, phospholipids and PM2 phages were dispersed in buffer B1 (1 M NaCl, 10 mM $CaCl_2$ and 10 mM Tris–HCl, pH 7.2) and in buffer B2 (2 M NaCl, 10 mM $CaCl_2$ and 10 mM Tris–HCl, pH 7.2) containing 60% sucrose, respectively. In the case of the host cells, the centrifuged pellet was used for the NMR measurements. Cross-polarization ^{31}P-NMR spectra were obtained with a JEOL FX-100 NMR spectrometer equipped with a solid-state NMR system at 40.3 MHz. The field was locked on external deuterium oxide (^2H_2O). A probe head specially designed for high-power and variable-temperature measurements was used, in which the sample tube is held vertically. A 3.6 s relaxation delay was used for the cross-polarization pulse sequence. The spin locking field was in the range of $4–7 \times 10^{-4}$ T. The proton spins were irradiated with the same power during data acquisition. Temperature calibration for the thermal contact time and proton decoupling was carried out. The number of data points used for a spectral width of 50,000 Hz was 4096. Phosphoric acid, 85%, was used as an external standard. The measurements of the spin-lattice relaxation time of proton in the rotating frame ($T_{1\rho}$(H)) was carried out by the use of the cross-polarization pulse sequence proposed by Stejskal et al. [11].

3. Results

Since slow motions are important to characterize the dynamic state of the biomembranes, the spin-lattice relaxation time of proton in the rotating frame ($T_{1\rho}$(H)) is a useful parameter to investigate the biomembranes. $T_{1\rho}$(H) of a single phosphorus component system such as a phospholipid bilayer can be obtained by the analysis of the contact time dependence of the integrated intensity of a cross-polarization spectrum as shown in the previous works [3,4,8]. However, this method cannot be used to obtain $T_{1\rho}$(H) of each component in an intact system. Because an intact system usually contains more than two phosphorus components, and the contributions from different components overlap each other in the spectrum. In the case of such a complicated system, the spectrum of each component should be separately observed. We showed that the ^{31}P-NMR spectrum of the biomembranes in intact systems can be selectively observed by taking advantage of the cross-polarization pulse sequence [3]. For example, a typical axially symmetric powder pattern of the ^{31}P-NMR spectrum of the biomembrane was selectively observed with the 6 ms thermal contact for the intact PM2 phage as shown in Fig. 1(A). Here, the spectra were measured in the presence of 60% sucrose to suppress the rotation of the phage particles. By fixing the thermal contact time and changing the spin-locking time of the proton spins preceding the thermal contact, we can observe the effect of $T_{1\rho}$(H) on the intensity of the cross-polarization spectrum [8]. For a

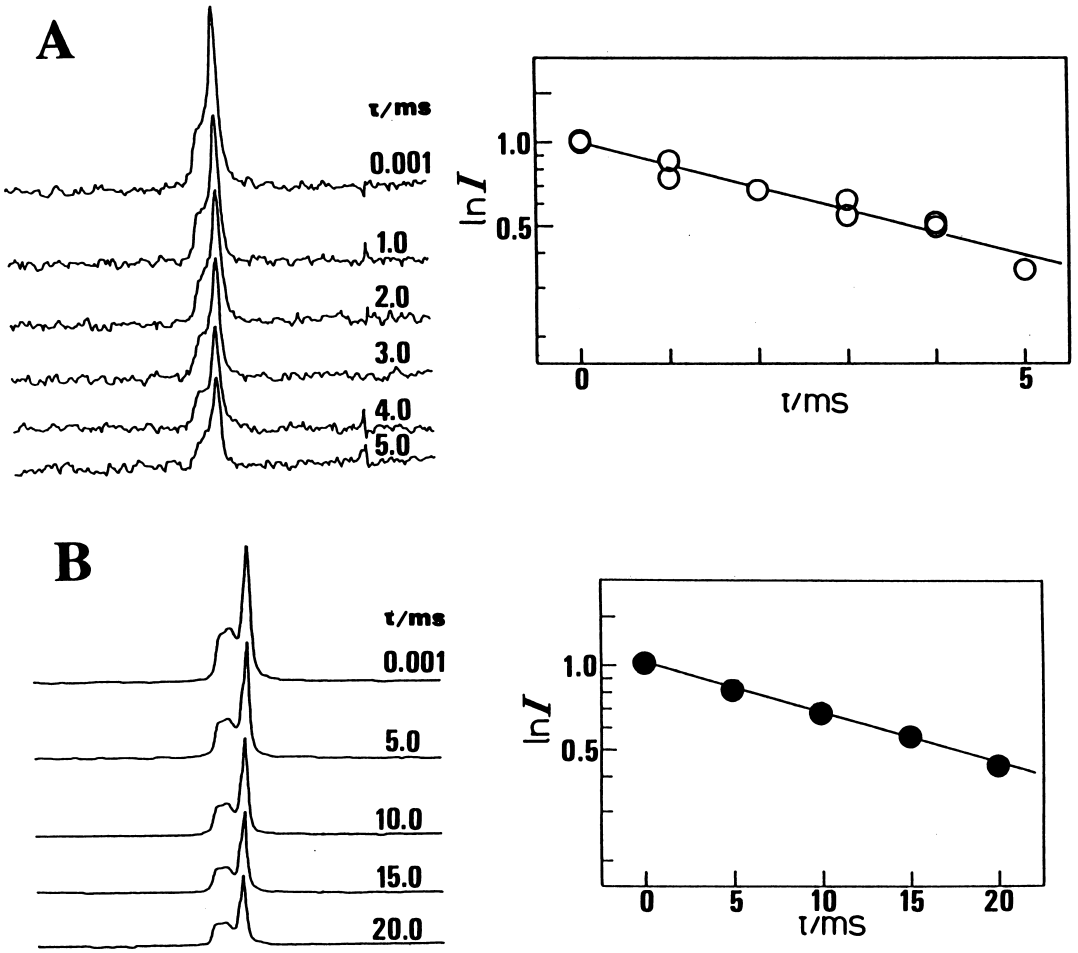

Fig. 1. The cross-polarization ^{31}P-NMR spectra of the PM2 membranes and semilogarithmic plots of the integrated intensity as a function of the proton spin-locking time τ preceding thermal contact at 4°C. A, the intact bacteriophage PM2 in buffer B-2 containing 60% sucrose, and B, the semi PM2 total phospholipid bilayers (PM2 PL) in buffer B-1. On the left, ^{31}P-NMR spectra obtained by the cross-polarization with various intervals τ. The thermal contact time was 6 ms for A and B. $T_{1\rho}$(H) can be obtained from the slope.

constant thermal contact time, the magnetization of the phosphorus nuclei can be described as follows:

$$M_P(\tau) = C \exp\left(-\tau/T_{1\rho}(H)\right), \quad (1)$$

where $M_P(\tau)$ is the magnetization transferred by cross-polarization with a delay time τ preceding the thermal contact, and C is constant. By choosing an appropriate constant thermal contact time included in the constant C, the spectrum of each component can be separately obtained [3].

The cross-polarization spectra of the intact PM2 phage, the host cells and the extracted phospholipid (phosphatidylethanolamine (PE), phosphatidylglycerol (PG), the *A. espejiana* total phospholipid (PE/PG = 75/23, referred to as cell PL hereafter), and the semi PM2 total phospholipid (PE/PG = 23/64, PM2 PL hereafter)) bilayers were measured as a function of the spin-locking time without thermal contact (τ). The used thermal contact times are 6 ms for the PM2 phage and the extracted phospholipids, and 7 ms for the intact host cells [3]. The integrated intensity of the cross-polarization spectrum of the bilayers of the intact PM2 phage and PM2 PL at 4°C is plotted as a function of τ in Fig. 1(A) and (B), respectively. The lineshape of the powder pattern did not change with the increase of τ, suggesting that $T_{1\rho}$(H) is isotropic in these systems. A single exponential was assumed because semilogarithmic plots

showed good linearity for most observations. This suggests that the heat reservoir of the proton spins in the biomembrane is simple in spite of its heterogeneous composition. The data were fitted to Eq. (1) by the linear least squares method. The best fits are shown by the solid lines. Obtained $T_{1\rho}$(H) of the PM2 phage and PM2 PL at 4°C were 4.8 and 25 ms at $5 \sim 6 \times 10^{-4}$ T of effective field in the rotating frame, respectively. The results for all membranes at 4°C are summarized in Table 1. The PE bilayers showed the shortest $T_{1\rho}$(H) among the extracted phospholipid bilayers. $T_{1\rho}$(H) became longer with the increase of PG content. Another remarkable point is that all of the extracted phospholipid bilayers showed longer $T_{1\rho}$(H) than the intact biomembranes.

The temperature dependence of $T_{1\rho}$(H) was examined extensively for the intact PM2 membrane and the extracted lipid bilayers. $T_{1\rho}$(H) of the intact PM2 phage is plotted as a function of temperature in Fig. 2. It was 4.0 ms at 0.5°C, and became very gradually longer with the increase of temperature, namely, with the increase of motion, up to about 18°C. It suggests that the relevant motion of the membrane is in the fast-motional regime. The $T_{1\rho}$(H) value changed significantly from 18 to 22°C, suggesting that a change in the motional state of the membrane took place in the region from 18 to 22°C. This result is in good agreement with the temperature dependence of the chemical shift anisotro-py, which was attributed to the phase transition of the lipid membrane of the PM2 phage [3]. Since the chemical shift anisotropy decreased in this temperature region, the increase of $T_{1\rho}$(H) should reflect an increase of the motion in the fast motional regime. On the other hand, $T_{1\rho}$(H) of the membrane of the intact *A. espejiana* cell was 9.8 ms at 10°C, which is longer than that at 4°C. It can be concluded that the relevant motion of the membrane of the intact *A. espejiana* cell is also in the fast-motional regime.

Table 1
The spin-lattice relaxation time of proton in the rotating frame ($T_{1\rho}$(H)) of phospholipids in various membranes at 4°C

Sample	90° pulse/μs	$B_{1H}/10^{-3}$ T	$T_{1\rho}$(H)/ms
Intact PM2 phage	12.5	0.47	4.8 ± 1.1
Intact host cells (*A. espejiana*)	12.5	0.47	8.6 ± 2.9
PE	10.2	0.58	11 ± 1
Cell PL (PE/PG = 75/23)	10.2	0.58	22 ± 3
PM2 PL (PE/PG = 23/64)	9.6	0.61	25 ± 1
PG	9.5	0.62	31 ± 2

$T_{1\rho}$(H) was obtained from the $^{1}H-^{31}P$ cross-polarization spectra of each membrane. PE, phosphatidylethanolamine; PG, phosphatidylglycerol; Cell PL, the total phospholipid extracted from *A. espejiana*; PM2 PL, a mixture of PG and PE at the ratio found in PM2 phage (semi PM2 total phospholipid). PE and PG were obtained from *A. espejiana* cells. B_{1H}, the magnetic field in the rotating frame of the proton spins.

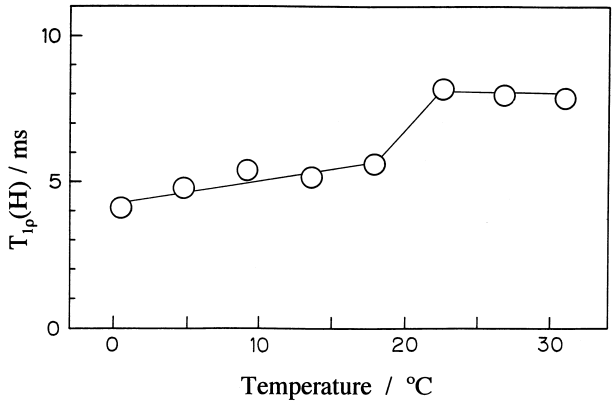

Fig. 2. Temperature dependence of the spin-lattice relaxation time of proton in the rotating frame ($T_{1\rho}$(H)) of the membrane of the intact PM2 phage.

The relaxation times of the extracted phospholipid bilayers are plotted as a function of temperature in Fig. 3. $T_{1\rho}$(H) of PE is 10 ms at 0°C, and became gradually longer with an increase of temperature, and reached 23 ms at 24°C. Cell PL and PM2 PL bilayers showed similar temperature dependence of $T_{1\rho}$(H) to each other, namely, 17 and 18 ms at 0°C, and 72 and 74 ms at 32°C, respectively. The PG bilayers showed the largest change of $T_{1\rho}$(H) among the extracted phospholipid bilayers. The relaxation time increased from 22 to 133 ms on raising the temperature from 0 to 34°C. Since all of their $T_{1\rho}$(H) became longer with an increase of temperature, the relevant motions are in the fast-motional regime in this temperature range. Although a discontinuous change according to the phase transition was observed in the temperature dependence of $T_{1\rho}$(H) for the intact PM2 membrane, it was not clear in those for the extracted phospholipid bilayers. This also suggests that the phase be-

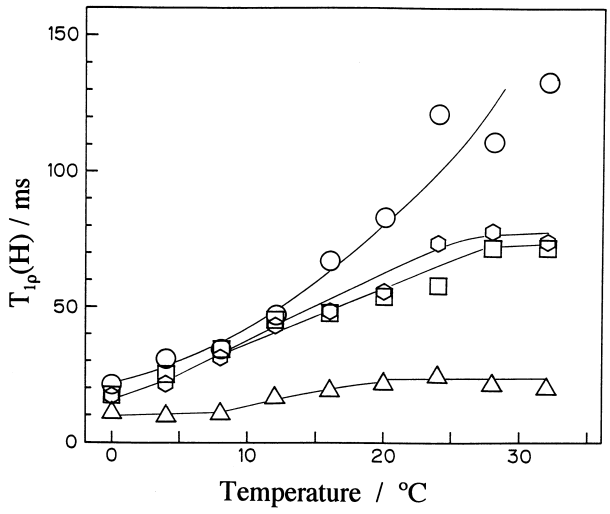

Fig. 3. Temperature dependence of the spin-lattice relaxation time of proton in the rotating frame ($T_{1\rho}$(H)) of the extracted phospholipid bilayers. The symbols are triangles, phosphatidylethanolamine (PE); squares, the *A. espejiana* total phospholipid (Cell PL); hexagons, the semi PM2 total phospholipid (PM2 PL); and circles, phosphatidylglycerol (PG).

havior of the intact PM2 membrane is different from those of the extracted phospholipid bilayers.

4. Discussion

The selective observation of the ^{31}P membrane spectra of the intact PM2 phage and its host cell, *A. espejiana*, by the application of the ^{1}H–^{31}P cross-polarization method enabled us to measure their $T_{1\rho}$(H) directly. It should be noted that the motion which dominates $T_{1\rho}$(H) is not necessarily restricted to the polar head group, because the proton spin temperature is common almost in the entire region of the membrane [8]. The motion most efficient for the spin-lattice relaxation in the rotating frame should determine $T_{1\rho}$(H). This parameter can provide us with a new information on the dynamics of the PM2 phage and *A. espejiana* in vivo which is different from the bulk property of the membrane obtained from the phosphorus chemical shift anisotropy.

The temperature dependence of $T_{1\rho}$(H) indicated that the relevant motions of the phospholipid molecules in all of the intact biomembranes and the extracted phospholipid bilayers are in the fast-motional regime ($\tau_c < 1.7 \times 10^{-6}$) at above 0°C. The axially symmetric powder patterns of ^{31}P-NMR spectra shown in Fig. 1 means that the phospholipid molecules in the membranes undergo a rapid axially symmetric rotation around a molecular axis (perpendicular to the membrane surface in average). Campbell et al. have analyzed the relationship between the lineshape of ^{31}P-NMR spectrum and the correlation time of the polar head group of phospholipid in a lipid bilayer [12]. They estimated that the correlation time of the rotational motion of the polar head group is shorter than 2×10^{-6} s when the powder pattern is axially symmetric. Thus, both time scales of the motions mentioned above are in good agreement with each other. Actually, it was suggested that the molecular rotation, specifically, the rotation of the glycerol backbone is responsible for the relaxation mechanism of $T_{1\rho}$(H) in the lipid bilayers [8]. The interaction between a membrane protein and lipid molecule would suppress the rotational motion of the glycerol backbone directly and indirectly. This can be responsible for the change of $T_{1\rho}$(H) in the intact membranes.

Since all of $T_{1\rho}$(H) of the intact biomembranes and the extracted phospholipid bilayers are in the fast-motional regime, we can compare their mobility qualitatively by referring to $T_{1\rho}$(H) at the same temperature provided that the second moments are similar to each other. In such a comparison, the effective field in the rotating frame should also be taken into account, since $T_{1\rho}$(H) depends on it. The result summarized in Table 1 indicates that the motion of the phospholipid molecules in the intact PM2 membrane is most suppressed among these lipid membranes, and that the mobility increases in the order of intact PM2, intact *A. espejiana*, PE, cell PL, PM2 PL and PG. This order agrees with the order of the temperature dependency of $T_{1\rho}$(H) of these membranes. The temperature change was the smallest for the PM2 phage and the largest for PG. Since temperature dependence of $T_{1\rho}$(H) should be smaller for the slower motion in the vicinity of the minimum of $T_{1\rho}$(H), this order seems to be reasonable. The order indicates that the relevant motions in the intact bio-membranes are more suppressed than those in the extracted phospholipid bilayers. Consequently, the suppressed motions should be attributed to the lipid–protein interactions.

The order of the mobility in the extracted phospholipid bilayers is reasonable. It is known that PE which

is a zwitter ionic phospholipid, forms salt bridges through hydrogen bonding among the polar head groups. In contrast, PG has a net negative charge in its head group so that the electrostatic repulsion is working among their head groups. Therefore, if the fatty acid composition is the same, PE bilayers are expected to be more rigid than PG bilayers, which is actually evidenced by the higher phase transition temperature of PE than PG. In Fig. 3, the values of $T_{1\rho}$(H) and the temperature dependence are quite similar for PM2 PL, cell PL and PG bilayers. It suggests that the effect of PG on the PE dynamics is not linear. Judging from Fig. 3, the presence of 30% PG is enough to change the nature of the PE dynamics significantly. It was reported that PE and PG took the uniform conformation in the region from the glycerol backbone to phosphate group because of the strong interaction among them [13]. This specific interaction explains, at least partially, the efficient effect of PG in changing the nature of the PE dynamics.

On the other hand, it was shown that the intact PM2 membrane was more rigid than the intact *A. espejiana* membrane in spite of a higher PG content of the former (PE/PG = 23/64) than the latter (PE/PG = 75/23). The simple chemical composition and well-established structure of PM2 phage [14,15] allow us to discuss the relationship between the structure and the dynamics in more detail. PM2 is a spherical bacteriophage composed of four kinds of proteins (proteins I, II, III and VI), DNA and lipids. It has a nucleocapsid with a lipid membrane encapsulating the DNA–protein VI complex. Proteins I and II are the spike and coat proteins, respectively. Protein III is embedded in the lipid membrane. The arrangement of the lipid bilayer in the phage was reported to be asymmetric, namely, most of PE and PG are located in the inner and outer leaflets of the bilayer, respectively [16]. The basic coat protein II interacts with acidic PG on the outer leaflet of the membrane. Since $T_{1\rho}$(H) of the PE and PG bilayers showed quite different temperature dependence (Fig. 3), lipid–protein interactions either between PG and protein II or between (PE + PG) and protein III should be responsible for the relaxation mechanism of $T_{1\rho}$(H) of the PM2 phage membrane.

Anyhow, the motional state of the lipid–protein interactions in the PM2 phage has significantly changed in the temperature range from 18 to 22°C. A change in the motional state in the same temperature range was also observed for the bulk of the DNA and lipid membrane of the PM2 phage [3,17] suggesting that the motional state of the whole virus has changed in this temperature range. This is a kind of a cooperative change in the motional state of the whole phage particle. Such a high cooperativity would come from the highly symmetric arrangement of its coat proteins and phospholipids. It is known that the PM2 phage can be adsorbed to the host cell at 4°C but cannot infect the cell. A significant increase of its infectivity was observed in the temperature range from 15 to 23°C [17]. The cooperative enhancement of the motional state of the whole phage particle in the same temperature range will be the reason of the significant increase in the infectivity.

Although the molecular structure of the *A. espejiana* membrane is not yet known, a highly organized structure like PM2 would not be the case. However, the lipid–protein interaction will be responsible for the relaxation mechanism also in this case as indicated. Judging from the relaxation times, the lipid–protein interactions in the host cell membrane are much weaker than those in the PM2 membrane. This can be ascribed to the rigid structure of the phage particle. While there is no lateral diffusion of the proteins in the PM2 membrane, the proteins are moving in the host cell membrane. Instead of the highly asymmetric phospholipid localization, however, there should be microheterogeniety in the host cell membrane. The microheterogeneity of the membrane is one of the important factors in the interactions between the lipid membranes and proteins [18,19]. Such microheterogeniety would affect $T_{1\rho}$(H) of the intact host cell membrane as well.

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