Hyperfine Splitting of 2,2,6,6-Tetramethylpiperidine-N-oxyl in Octyl β-D-Glucoside-Containing Large Unilamellar Vesicle Suspensions

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Hyperfine splitting patterns of the ESR spectra of 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) in octyl β-D-glucoside (OG)-containing egg yolk phosphatidylcholine (EPC) large unilamellar vesicle suspensions were mainly composed of TEMPO dissolved in water, in lamellar EPC, and in nonlamellar EPC. Their hyperfine splitting constants and half widths were available for monitoring the behavior of OG in the course of vesicle destruction.

2,2,6,6-Tetramethylpiperidine-N-oxyl (TEMPO) is a spin label which is soluble both in water and in lipid. Because of the difference in hyperfine splitting constants between TEMPO dissolved in water and in lipid, two components were observed in the I = +1 hyperfine splitting bands.[1-3] On the other hand, we have studied on the micelle-vesicle transition in order to find out the size-determining factors of egg yolk phosphatidylcholine (EPC) vesicles with the aid of electron micrography and particle sizes of detergent-containing vesicles, partition coefficients of detergents between water and lipid, and so on.[4,5] Consequently, following four stages occurred with an increase in detergent concentration: I) distribution of detergents into vesicles in the first stage of the vesicle destruction, II) transition to small vesicles containing high amount of detergents (SUV*) in the second stage, III) transformation to intermediate structures in the third stage, and IV) formation of mixed micelles in the fourth stage.[5,6] We recently obtained electron spin resonance (ESR) spectra of TEMPO in octyl β-D-glucoside (OG)-containing large unilamellar vesicles (LUV). Their hyperfine splitting patterns were significantly different from those of TEMPO in detergent-free lipid suspensions.[1-3] In the present paper, we will explore the source of this spectral change and examine the behavior of TEMPO in OG-containing LUV in relation to the micelle-vesicle transition using hyperfine splitting constants and half widths of TEMPO dissolved in membrane as parameters.

The suspension of LUV was prepared by extrusion. Its diameter was estimated to be 200 nm by quasielastic light scattering experiments. After the addition of OG and TEMPO (1 mol% lipid concentration (40 mM)) to this suspension, ESR spectra were recorded on a JEOL JES FE3X type spectrometer at 25 °C. Figure 1 depicts I=+1 hyperfine splitting bands of TEMPO dissolved in 0, 40, 70, 100, 130, 180, and 240 mM OG-containing LUV at 1d after the addition of OG and TEMPO. Other two (I=0, and -1) bands were unresolved. Phase transition of the LUV destructior occurred at OG concentrations of 24, 52, and 120 mM. Phases predominantly appearing in the lipid systems are also shown in Figure 1. ESR spectra in Figures 1A-C are almost the same as, while those in Figures 1E-G are significantly different from the well-known spectra of TEMPO dissolved in lipid suspensions.[1-3]

Next, ESR spectra obtained at 1 d after the addition of

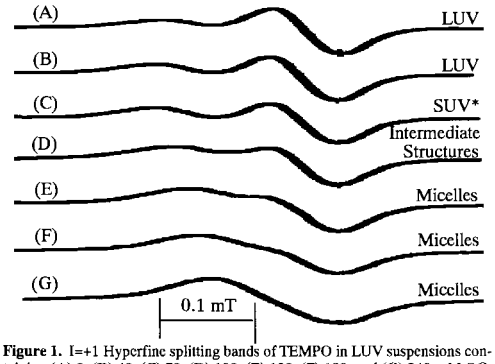

Figure 1. I=+1 Hyperfine splitting bands of TEMPO in LUV suspensions containing (A) 0, (B) 40, (C) 70, (D) 100, (E) 130, (F) 180, and (G) 240 mM OG. Phases predominantly appearing in the LUV systems are also shown.

TEMPO were applied to the study of the LUV destruction by OG. Usually, the TEMPO parameters have been employed for monitoring, for example, phase transition points from the inflection points appearing in the TEMPO parameter-temperature relationship.[3] In the present paper, we employed hyperfine splitting constants (A) and half widths (δH) of total membrane components, obtained by subtracting the spectrum of TEMPO in water (actually, a buffer solution (20 mM TES, 250 mM NaCl, 1 mM EDTA; pH 7.0)) from the observed spectra as easily obtainable parameters for probing the behavior of TEMPO in LUV. We will describe below in this paper that the total membrane components are made up of three components. Figure 2 shows the dependence of (A) A (obtained from the I=-1 and +1 bands) and (B) δH (obtained from the I=+1 band) on OG concentration. Since the values of A of TEMPO in water, methanol, OG-free LUV, and heptane were 1.75, 1.62, 1.64, and 1.54 mT, respectively, TEMPO exists in the hydrophilic part of EPC. Inflection points appearing in Figure 2A almost agree with the phase transition points of the LUV destruction. In the first and second stage of the LUV destruction, the values of A decrease, indicating that TEMPO is transferred to less polar part near the surface of EPC, which is induced directly by the added OG. In the third stage, the A values increase with an increase in OG concentration. As lamellar structure of EPC is destroyed, TEMPO moves to more polar part of the membrane. Next, as can be seen from Figure 2B, in the first and second stages of the vesicle destruction the

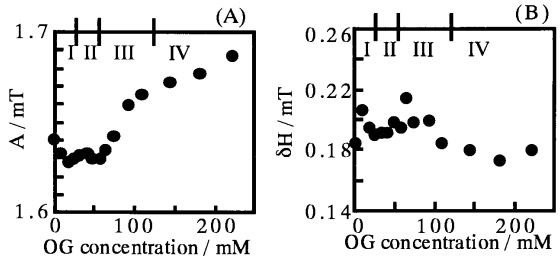

Figure 2. Dependence of (A) hyperfine splitting constants (A) and (B) half widths (δH) of total membrane components on OG concentration.

Table 1. Values of g, A, and δH of TEMPO in various solvents

Solvent	g	A / mT	δH / mT
membrane 1[a]	2.0069	1.64	0.18
membrane 2[b]	2.0065	1.63	0.12
water[c]	2.0065	1.75	0.15
10 mM OG solution	2.0058	1.69	0.27
20 mM OG solution	2.0058	1.69	0.26
30 mM OG solution	2.0058	1.69	0.25

[a] Previously known membrane component obtained with OG-free LUV. [b] New membrane component obtained with OG-free LUV. [c] Actually, a buffer solution (20 mM TES, 250 mM NaCl, 1 mM EDTA; pH 7.0).

δH values increase with increasing OG concentration due probably to the suppression of movement of TEMPO induced by OG. As will be described below, δH increases in the first half and decreases in the latter half of the third stage. When sodium cholate or octaethyleneglycol mono-(n-dodecyl) ether, instead of OG, was used for the LUV destruction, the behavior of A and δH was strongly dependent on the kinds of detergents. Further details will be described elsewhere. Thus, A and δH are proved to be useful parameters for probing the behavior of TEMPO in EPC vesicles.

Next, we attempted to explore the source of the spectral variation (Figure 1). In the first place, LUV suspension was prepared differently by using TEMPO-containing EPC having once been dried under reduced pressure (ca. 350 Pa) for more than 10 h. Although the content of TEMPO was small (less than 0.1 mol% lipid concentration), an ESR spectrum of TEMPO in OG-free LUV was observable. Subtraction of the spectrum of TEMPO dissolved in water from the observed spectrum, obtaining a 1:1:1 hyperfine splitting pattern of TEMPO in membrane without any detergent, which was significantly different from that reported previously.[1-3] The g value (g), A, and δH of this membrane component (named membrane 2 (m_2) component) as well as those of TEMPO dissolved in water (w) and in membrane 1 (m_1: previously known membrane component) are tabulated in Table 1. The values of the m_1 component were obtained by subtracting the w component from the spectrum of an OG-free LUV (Figure 1A). Small δH value of the m_2 component may be caused by the small amount of TEMPO in LUV. The results of TEMPO dissolved in 10, 20, and 30 mM OG solution (or micelles) (c.m.c = 22 mM) are also listed in Table 1, indicating that the values of g and A are independent of OG concentration from 10 to 30 mM. The spectra of TEMPO in the detergent (d component) are characterized by a large value of δH. Since TEMPO is somewhat volatile, the spectral intensities decreased with the passage of time when the LUV suspension were left at 25 °C under nitrogen atmosphere. Although the amount of m_2 component was too small to be observed at 1 d after the addition of TEMPO (Figure 1A), it became observable within 7 d. Anyway, the 1:1:1 hyperfine splitting pattern of TEMPO in m_2 without any m_1 component was obtained by using the TEMPO-containing LUV having once been dried under reduced pressure.

The values of g and A of m_1 and m_2 components in the presence of OG may vary with the polarity change around TEMPO. Assuming a spectrum of TEMPO dissolved in membrane (obtained by the subtraction of w component from the observed spectrum) to be the sum of three Lorentzian (m_1, m_2, and d (obtained by subtracting the w component from the spectrum of TEMPO in 30 mM micellar solution)), the curve fitting (Levenberg-Marquardt method) of the spectrum shown in Figure 1 was carried out. Consequently, the g values of m_1 and m_2 components of OG-containing LUV were almost identical with those of OG-free LUV (Table 1), while A values of the $m_1$and m_2 components varied with the polarity change induced by OG from 1.63 to 1.68 and from 1.63 to 1.69, respectively. The value of δH of m_2 component of OG-containing LUV (0.18 mT) was much greater than the value listed in Table 1 and became comparable to that of the m_1 component. The d components were easily separated from other (m_1 and m_2) membrane components due to their large δH values. Thus, the variation in spectral shapes in Figure 1 was largely responsible for the difference in g values between the m_1 and m_2 components.

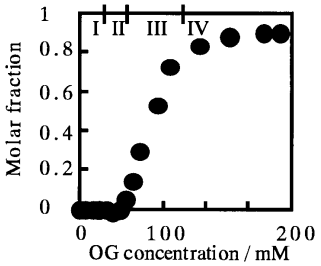

Figure 3. Plots of molar fraction of m_2 against OG concentration. Molar fraction of m_2 is defined as moles of TEMPO in m_2 / moles of TEMPO in total membrane components (m_1, m_2, and d).

Figure 3 exhibits the plots of molar fraction of TEMPO dissolved in m_2 against OG concentration. The molar fraction is defined as moles of TEMPO in m_2 / moles of TEMPO in total membrane components (m_1, m_2, and d) evaluated from the spectra in Figure 1. As can be seen from Figure 3, only m_1 exists in the first and second stage of the LUV destruction measured at 1 d after the addition of OG and TEMPO to the LUV suspension. In the third stage, the amount of m_1 decreases, while that of m_2 increases with increasing OG concentration. In the fourth stage, membrane component is mainly composed of m_2. Even in this stage, small amount of m_1 still remains, indicating that the arrangement of EPC molecules in the mixed micelles are somewhat ordered at a low OG concentration. The content of d component increases with an increase in OG concentration, reaching almost the difference between 1.0 and the observed molar fraction at the highest OG concentration examined (240 mM). Next, as can be seen from Figure 2B, apparent half width of TEMPO in total membrane increases in the first half of the third stage because of the coexistence of two membrane components possessing different g values. In the latter half of the third stage, ESR spectra become gradually sharp with a decrease in the amount of m_1, with an increase in that of m_2, and with increase in the freedom of motion of TEMPO.

Judging from the LUV destruction mechanism described above, the m_1 and m_2 components were assigned to TEMPO dissolved in lamellar and nonlamellar EPC, respectively. Thus, the total membrane components of the ESR spectra of TEMPO were confirmed to be composed of three (m_1, m_2, and d) components.

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