The effect of vitamin E on the structure of membrane lipid assemblies

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Abstract **The effects of vitamin E on the activity of membrane-dependent enzymes suggest that it acts indirectly by modifying some properties of the lipid host. The effects of α-tocopherol (α-T) and α-tocopherol hemisuccinate (α-THS) on phospholipid monolayer structure, curvature, and bending elasticity were examined using X-ray diffraction and the osmotic stress method. These ligands were mixed with the hexagonal phase-forming lipid, dioleoylphosphatidylethanolamine (DOPE). Increasing levels up to 50 mol% α-T in DOPE in excess water result in a systematic decrease in the lattice dimension. Analysis of the structural changes imposed by α-T shows that it contributes a spontaneous radius of curvature of −13.7 Å. This unusually negative value is comparable to diacylglycerols. α-T does not affect the bending elasticity of these monolayers. α-THS in its charged form decreases membrane curvature, but in its undissociated neutral form has a qualitatively similar but reduced effect on monolayer curvature, as does α-T. We discuss these results in terms of the local stresses such ligands would produce in the vicinity of a membrane protein, and how one might expect proteins to respond to such stress.**—Bradford, A., J. Atkinson, N. Fuller, and R. P. Rand. **The effect of vitamin E on the structure of membrane lipid assemblies.** ***J. Lipid Res.*** **2003.** 44: **1940–1945.**

Supplementary key words α-tocopherol • spontaneous curvature • dioleoylphosphatidylethanolamine • α-tocopherol hemisuccinate

α-Tocopherol (α-T) has been shown to have two major roles in membranes since it was first discovered *1*) as a lipid-soluble antioxidant that acts to prevent free radical damage of polyunsaturated fatty acids (1–5), and *2*) as a membrane-stabilizing agent through its van der Waals interaction with membrane phospholipids. This latter ability to stabilize membranes may help to prevent the damaging actions of phospholipases, although this is still under debate (6–10). α-T has also been shown to inhibit protein kinase C (11–13), apparently without directly binding to the enzyme but rather by activating the translocation of the protein phosphatase 2A to the plasma membrane (14). These and other observations, such as those of the effect of α-T on diacylglycerol (DAG) kinase (15, 16), CoA-independent transacylase (17), and phospholipase D (18) suggest that the modulation of enzyme activity may have more to do with the effect tocopherol has on membrane structure, particularly inasmuch as all these enzymes act on substrates that are in membranes or are activated/inhibited when translocated to a membrane surface.

α-Tocopherol hemisuccinate (α-THS) also exhibits biological activity and has been implicated as a cancer chemopreventative agent with chemotherapeutic potential (19). It has been shown that this succinate derivative can induce apoptosis through a variety of pathways, such as G_1 cell blockage, DNA synthesis arrest, and activation of transforming growth factor β and enhanced expression of its type II receptor (20). α-THS differs from the parent molecule by having a succinic acid moiety esterified to the chroman phenol, and it is this modification that eliminates the classical antioxidant activity of α-T. A related ester, cholesterol hemisuccinate, is known to interact with membrane components, reducing acyl chain mobility and increasing the surface charge (21).

Because α-T and α-THS may alter enzyme activity by changing the biophysical properties of the membrane, it is of interest to determine what effect the addition of these compounds has on the structure of lipid assemblies. To investigate the effect of α-T and α-THS on such assemblies, we report here the use of X-ray diffraction to characterize the structures formed by hydrated lipid membranes (22). Hubner et al. (23) and others (24–26) have shown that curvature affects the activity of membrane enzymes in vesicles. We have measured the influence of α-T and α-THS on the spontaneous curvature and bending modulus (K_{cp}) when added to dioleoylphosphatidylethanolamine (DOPE) lipid monolayers. Among the multitude of heterogeneous membrane sites, such model systems as explored here are intended to reflect only the properties of specific membrane sites that contain α-T.

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Abbreviations: α-T, α-tocopherol; α-THS, α-tocopherol hemisuccinate; DAG, diacylglycerol; DCG, dicaprylglycerol; DOG, dioleoylglycerol; DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; H_{II}, hexagonal phase; K_{cp}, bending modulus.

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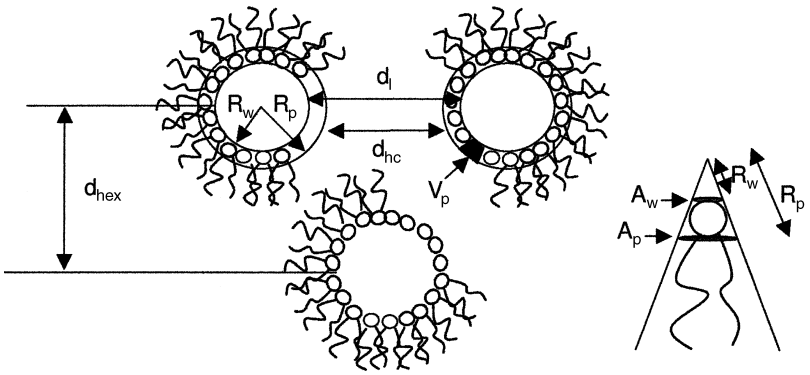

Fig. 1. Schematic representation of hexagonal phase (H_{II}) structural parameters.

MATERIALS AND METHODS

Sample preparation

α-T was obtained by hydrolysis (MeOH, K_2CO_3) of commercially available natural source α-tocopheryl acetate and was purified by silica gel column chromatography before use. α-THS was obtained from Sigma Aldrich Canada (Oakville, Ontario) and was used without further purification. The sodium salt of α-THS was prepared by titration of a diethyl ether solution of a α-THS with NaOH in EtOH. The resulting precipitate was rinsed with ether-EtOH (20:1; v/v) and dried in vacuo. Synthetic DOPE was purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. DOPE and α-T were stored under nitrogen at −18°C. α-THS was stored at room temperature, and all water used in experiments was double distilled.

The desired lipid mixtures were produced by combining the required amounts of DOPE with either α-T or α-THS in chloroform solution. The chloroform was then removed by rotary evaporation under a constant stream of nitrogen, followed by vacuum desiccation. The dry lipid mixtures were hydrated to varying degrees by gravimetric addition of double-distilled water or excess amounts of polyethyleneglycol solutions of different measured osmotic pressures. The hydrated samples were then given another 72 h to equilibrate at room temperature. The equilibrated samples were then mounted between two mica windows with powdered teflon (as an X-ray calibration standard, repeat spacing of 4.87 Å) and examined by X-ray diffraction.

X-ray diffraction

To characterize the structures formed by the final hydrated lipid mixture, an X-ray diffraction method was used. A Rigaku rotating anode generator produced a CuKα1 line (λ = 1.540 Å), which was isolated using a bent quartz crystal monochromator. The diffraction patterns of the hydrated lipid were photographed using Guinier X-ray cameras. The temperature of the sample was controlled by thermoelectric elements and maintained at 22°C ± 0.5°C. Samples that formed hexagonal phases (H_{II}s) are characterized by at least three X-ray spacings with the ratios to the dimension of the first order [hexagonal lattice dimension (d_{hex})] of 1, $1/\sqrt{3}$, $1/\sqrt{4}$, $1/\sqrt{7}$, $1/\sqrt{9}$, $1/\sqrt{12}$, etc. Lattice dimensions of the hexagonal structures could be measured to ±0.1 Å on any one sample.

Structural analysis

H_{II}s are two-dimensional hexagonal lattices with the water cores centered on the prism axes and lined with lipid polar groups, while the rest of the lattice is filled with the hydrocarbon chains. For an H_{II} of known composition, its lattice can be divided into compartments, as shown in **Fig. 1**, each containing the volume fractions of lipid and water. This average division follows the method originally introduced by Luzzati and Husson (27) and depends only on knowledge of the specific volumes of the molecular components and their linear addition. Some of the physicochemical and structural parameters for the lipid components used in this study are listed in **Table 1**.

The water and lipid components can be separated through the introduction of an idealized cylindrical interface, the Luzzati surface, in which all of the water is inside this cylinder and all of the lipid is outside. The radius of this water cylinder, R_w, is related to both the volume fraction of the water in the sample, ϕ_w, and d_{hex}, as follows in equation 1.

$$R_w = d_{hex}\sqrt{\frac{2\phi_w}{\pi\sqrt{3}}} \qquad \textit{(Eq. 1)}$$

The area per lipid molecule at the Luzzati surface is given in equation 2 as:

$$A_w = \frac{2\phi_w V_1}{(1-\phi_w)R_w} \qquad \textit{(Eq. 2)}$$

where V_1 is the volume of the lipid molecule. ϕ_w is calculated from the weight fraction of water using the specific volumes stated in Table 1.

When the volume of a lipid molecule is used it is based on the notion of an effective molecule, i.e., one phospholipid, DOPE of volume V_{pl}, plus x-(α-T) molecules, where $V_{\alpha\text{-}T}$ is the volume of α-T and x is the molar ratio of α-T to phospholipids. The effective molecular volume is then given in equation 3 as:

$$V_1 = V_{p1} + xV_{\alpha-T} \qquad \textit{(Eq. 3)}$$

The molecular area (A) at, and the radius (R) of, any cylindrical dividing surface separated from the Luzzati surface by volume (V), are given in equations 4 and 5 as:

TABLE 1. Physiochemical and structural parameters of molecules used in this study

	Molecular Mass	Density	Specific Volume
		g/cm^3	cm^3/g
Dioleoylphosphatidylethanolamine	744.0	1.00	1.00
α-T	430.7	0.95	1.05
α-THS	530.8	0.95[a]	1.05

α-T, α-tocopherol; α-THS, α-tocopherol hemisuccinate.
[a] Density of α-THS derivative is an approximation based on the density of α-T acetate.

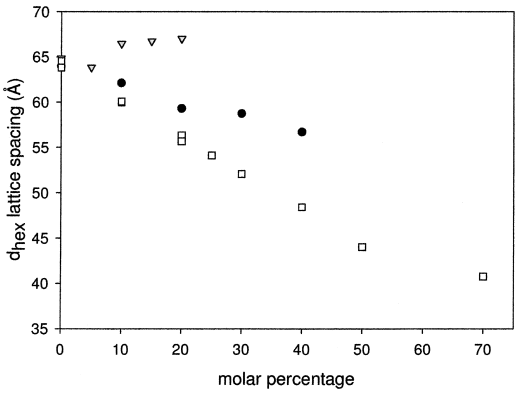

Fig. 2. Plot of the equilibrium lattice spacing, hexagonal lattice dimension (d_{hex}), for lipid mixtures of increasing amounts of α-tocopherol (α-T) (square), α-tocopherol hemisuccinate (α-THS) (circle), and α-T sodium-succinate (triangle) in dioleoylphosphatidylethanolamine (DOPE).

$$A = A_w\sqrt{1+\frac{1-\phi_w}{\phi_w}\frac{V}{V_1}} \qquad \textit{(Eq. 4)}$$

$$R = R_w\sqrt{1+\frac{1-\phi_w}{\phi_w}\frac{V}{V_1}} \qquad \textit{(Eq. 5)}$$

To determine if there is a surface of constant area, i.e., a pivotal plane, equation 4 can be expressed in a form that uses normalized areas and volumes (28) (equation 6):

$$\frac{A_w^2}{V_1^2} = \frac{A_p^2}{V_1^2} - 2\frac{V_p}{V_1}\left(\frac{A_w}{V_1 R_w}\right) \qquad \textit{(Eq. 6)}$$

If a straight line results from plotting $(A_w/V_l)^2$ versus $A_w/(V_lR_w)$ (our "diagnostic plot"), then the system has a dividing surface that has a constant molecular area and is defined as the pivotal plane. The slope (V_p/V_l) of the diagnostic plot gives the position of the pivotal plane. Equation 5 can then be used to calculate the radii of curvature (R_o) of the H_{II} monolayers. At full hydration, $R = R_{op}$, the spontaneous curvature of the lipid monolayers. Once R_{op} has been determined for the mixed monolayer, the intrinsic curvature of the individual lipids can also be determined using equation 7, if it is linear:

$$\frac{1}{R_{0p}} = (1-m_{\alpha-T})\frac{1}{R_{0p}^{DOPE}} + m_{\alpha-T}\frac{1}{R_{0p}^{\alpha-T}} \qquad \textit{(Eq. 7)}$$

where the molar fraction α-T is given by $m_{\alpha-T} = x/(1+x)$ (28).

Once the pivotal plane is known, the elastic free energy, F, of the H_{II} can be approximated by the energy of bending (29, 30), as shown in equation 8.

$$F = \frac{1}{2}K_{cp}A_p\left(\frac{1}{R_p}-\frac{1}{R_{0p}}\right) \qquad \textit{(Eq. 8)}$$

where K_{cp} is the bending modulus, A_p is the molecular area, and R_p and R_{0p} are the R_o and the spontaneous R_o respectively.

By comparing the elastic free energy of the lipid mixture (equation 8) under conditions of osmotic stress, with the osmotic work done by the osmotic stress (Π), a plot of (ΠR_p^2) versus $(1/R_p)$ gives, from the slope, the monolayer K_{cp} (31, 32), as shown in equation 9.

$$\pi R_p^2 = 2K_{cp}\left(\frac{1}{R_p}-\frac{1}{R_{0p}}\right) \qquad \textit{(Eq. 9)}$$

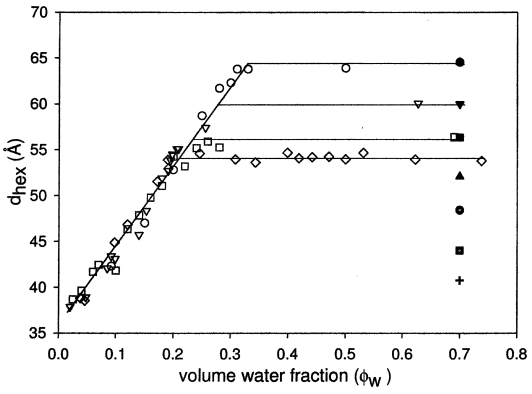

Fig. 3. Lattice dimension, d_{hex}, as a function of water content for the H_{II} formed by mixtures containing DOPE and the indicated mole% α-T: 0% (open circle); 10% (open triangle); 20% (open square); and 25% (open diamond). In a separate experiment (closed symbols), the trend in lattice dimension change with α-T content, and at full hydration was more accurately determined by systematic serial dilution of lipid mixtures: 0% (closed circle); 10% (closed inverted triangle); 20% (closed square); 30% (upright closed triangle); 40% (round ring); 50% (square ring); and 70% (cross).

RESULTS

The relationship between mol% α-T or α-THS in DOPE and the equilibrium lattice spacing of the resultant H_{II} is shown in **Fig. 2**. For both α-T and α-THS, the dimension of the H_{II} decreases with increasing tocopherol content. It appears that both α-T and α-THS are increasing the curvature of the mixed monolayers, α-T more so than α-THS.

Gravimetric phase diagrams covering the full hydration range for three mixtures of α-T with DOPE are shown in **Fig. 3**. The d_{hex} is shown as it varies with weight fraction lipid in water. d_{hex} for all the single H_{II}s increases with water content until a maximum is reached. That maximum depends on the α-T content. Several α-T/DOPE ratios were prepared and their maximally hydrated equilibrium hexagonal dimensions determined (Fig. 3). The weight

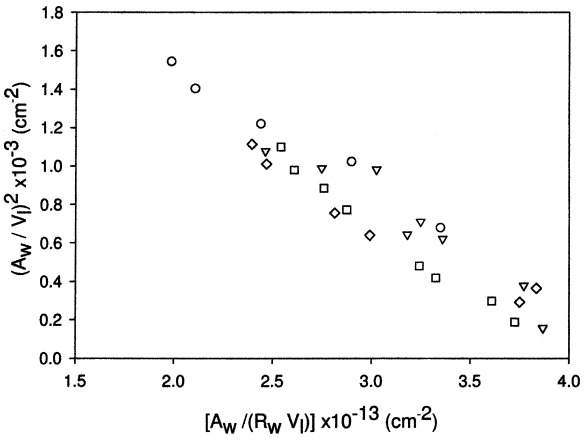

Fig. 4. Diagnostic plots for the lipid mixtures: 0% (circle); 10% (triangle); 20% (square); and 25% (diamond) of Fig. 3. Linearity indicates a pivotal plane, the slope of the line gives its position, V_p/V_l, and its intercept, A_p/V_l, gives the effective molecule (see Materials and Methods) at this plane.

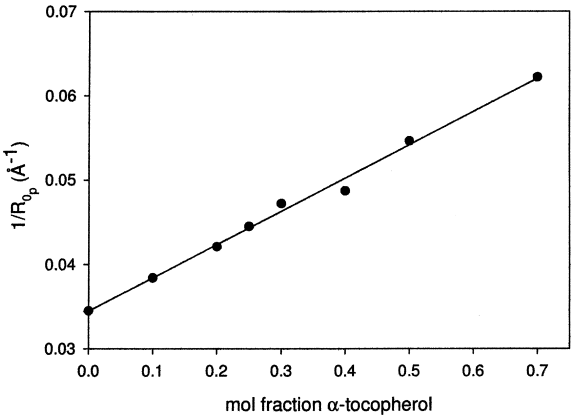

Fig. 5. Plot of spontaneous monolayer curvature, $1/R_{0p}$, for DOPE/α-T mixtures as a function of α-T content.

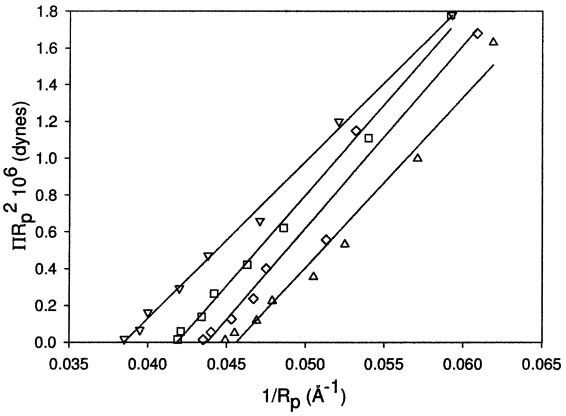

Fig. 6. Plot relating the osmotic work required to dehydrate the H_{II} of DOPE/α-T mixtures with the change in monolayer curvature $1/R_p$. The slope, determined by least-squares fit (all r values > 0.96), gives the measure of the bending modulus (K_{cp}): 15% (inverted triangle); 20% (square); 25% (diamond); and 30% (upright triangle).

fraction of lipid at the maximum hydration for every α-T/DOPE ratio was determined from the intercept of the best-fit curve below excess water, with the average maximum dimension in excess water.

Figure 4 shows the diagnostic plots of $(A_w/V_l)^2$ versus $A_w/(V_lR_w)$ for all α-T/DOPE mixtures. The linearity of this relationship indicates that there is a well-defined pivotal plane for all of these mixtures. Therefore, there exists a position in the monolayer that does not change area even as the monolayer is bent upon dehydration. The position of this pivotal plane is given by the slope of the relationship in Fig. 4, (V_p/V_l). V_p/V_l for α-T in DOPE is 0.32.

The spontaneous R_o of the lipid monolayers, R_{op}, is calculated from equations 1 and 5 using the equilibrium volume fraction in excess water, the maximum lattice dimension for d_{hex}, and the value of V_p/V_l. **Figure 5** shows that α-T increases monolayer curvature. The linear relationship allows the apparent spontaneous curvature of the individual lipids to be determined from equation 7. R_{op} for α-T is −13.7 Å, making it a membrane component with one of the most negative curvatures measured, in the category of the diacylglycerols, and considerably more negative than cholesterol. R_{0p} for DOPE is −29.4 Å, which is consistent with several previous studies (22, 27). These curvature values are shown in **Table 2**.

Following equation 9, **Fig. 6** shows the ΠR_p^2 versus $1/R_p$ plots, a measure of how easy it is to bend these monolayers as water is withdrawn osmotically. From the slopes of these plots, we are able to determine the K_{cp} of the α-T/DOPE mixtures. The K_{cp}, plotted in **Fig. 7**, show that α-T does not change the bending elasticity of these monolayers.

TABLE 2. Comparison of spontaneous radii of curvature and bending moduli for individual lipids

	R_o (Å)	K_c/kT	References
DOG (in DOPE)	−11.5		28
DOG (in DOPC)	−10.1		33
DCG (in DOPC)	−13.3		33
α-T (DOPE)	−13.7		
DOPE	−28.5	11	28
DOPE-tetradecane	−28.7	12	22
Cholesterol (in DOPE)	−22.8		22
Cholesterol (in DOPC)	−27.2 (32°C)		22

DCG, dicaprylglycerol; DOG, dioleoylglcerol; DOPC, dioleoylphosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; R_o, radius of curvature.

DISCUSSION

We have investigated the modifying effects of α-T on phospholipid structures with the hope that such measurements may lead to an understanding of the mechanism by which α-T affects the activity of membrane resident enzymes. Inasmuch as direct binding of α-T has not been demonstrated, it is possible that tocopherol(s) modify the physical properties of the protein's lipid milieu. Local curvature is one physical parameter that is thought to produce local stress in bilayer membranes and so affect protein-lipid interactions and, thereby, protein conformation and activity. One clear example is that of PI-3 kinase (23), whose activation appears to depend on the curvature of its host lipid vesicle. We have measured the contribution of several different types of lipid-to-membrane curvature.

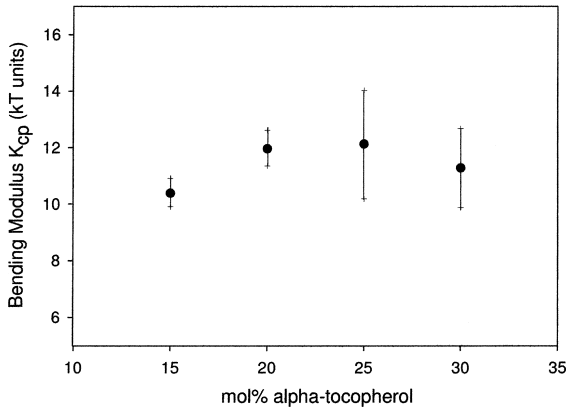

Fig. 7. K_{cp} calculated for increasing amounts of α-T in DOPE mixtures.

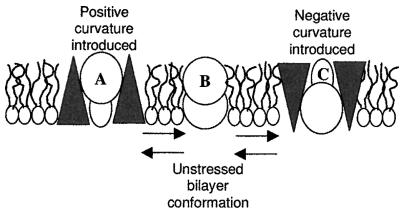

Fig. 8. The conformational changes that a membrane-bound enzyme (A, B, or C, large circles and connected ovals) might undergo to compensate for the addition of lipid with either positive (upright triangle) or negative (inverted triangle) curvature in its vicinity.

This is done by determining the stress free, or intrinsic, or spontaneous curvature of lipid assemblies. The higher this curvature, the more local stress a lipid will produce when confined to a flat bilayer membrane, and the higher the driving force in lipid–protein interactions. We have illustrated this hypothetical mechanism in **Fig. 8**.

Several enzymes are affected by α-T without direct binding (11–13), and that may be a result of a general effect tocopherol has on membrane structure. In this study, we have shown that α-T has one of the smallest intrinsic R_os, and therefore contributes one of the highest negative curvatures measured in membranes, comparable to that of the DAGs (33). According to our hypothesis, α-T's action on membrane-resident enzymes is through the local curvature it produces in the vicinity of these proteins. This curvature stress results from the addition of relatively more mass to the hydrocarbon portion of the membrane monolayers than to the polar portion. One might expect that proteins in the presence of α-T would react to this stress. Stress reduction would result from a conformational change involving a movement of protein mass from the hydrocarbon to the polar group region of the monolayer. This change is the example shown schematically in the transition (Fig. 8B–C).

α-T does not apparently affect the K_{cp} of the membrane monolayers. Such K_{cp} is relevant to any local deformation of the monolayer as might be required by the protein in adjusting to lipid-protein packing of the new conformation.

We could not make equivalent measurements for α-THS, but the qualitative effects that it has on local curvature appear clear. α-T sodium succinate increased the hexagonal lattice of DOPE, indicating the addition of a much lower spontaneous curvature than that of α-T, a likely consequence of charge repulsion at the polar group layer. The protonated form of α-THS, however, had the same, although smaller, effect on the DOPE lattice dimension as did α-T. Thus, it behaved as if it were uncharged (undissociated), and its larger polar group likely added less negative curvature than did α-T itself.

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