jz-2022-03098r.R1

Name: Peer Review Information for "Suppression of Amyloid-β Adsorption on Endoplasmic Reticulum Stress-Mimicking Membranes by α-Tocopherol and α-Tocotrienol"

First Round of Reviewer Comments

Reviewer: 1

Comments to the Author

The authors' major advance is that the modulation of VE on lipid membrane phases would lead to reduced adsorption of Amyloid-β to membranes and this is of major interest to the scientific field. The mechanism for the reduced AB absorption by VE was shown to be by inhibiting the formation of the So phase. Therefore it builds on the assumption that the ER would have solid ordered phases, as the ER connection is highlighted through the article as the important biological relevance of the findings. The solid-ordered phases in the ER seem to be plausible but the authors do not clearly communicate this. The connection between ER solid phases is not clear to most readers and needs to be explained carefully and the manuscript should be partially rewritten to clarify this. See details below:

The authors state "The ER membrane is known to have low Chol content and forms the So phase" and refers to references 17–19. References 17-18 confirm the low cholesterol concentration in the ER which enables the lipids to form a solid phase at low temperatures or in the presence of di-saturated phospholipids. In reference 19 it was interestingly and surprisingly shown that the addition of high concentrations of the saturated palmitate led to the synthesis of disaturated PCs in the ER leading to the formation of solid-ordered phases in the ER. Therefore, the authors' statement that ER forms the solid ordered phases should be rewritten as "may in special cases of ER stress form solid ordered phases". The authors need to highlight that ER can form solid phases only in these special circumstances (excess palmitate or other saturated FFA feed to cells) even if it is highly relevant for biological systems. It is also misleading to say that DOPC/DPPC membranes are "ER mimicking membranes", they should be stated as "ER stress mimicking membranes". These points need to be considered and rewritten throughout the whole text.

The authors have also used the fluorescent probe Rhodamine which is known to be prone to lipid oxidation. The authors do mention that the time exposure was limited, which is good. Still, it would be good to confirm some of the results with another Ld-sensitive fluorophore probe, e.g. FAST DiO or DiD-C18.

Minor:

The sentence "We hypothesized that the accumulation of Aβ on the So phase causes ER stress." in the introduction, could be better placed in the discussion/conclusion part because while likely the authors do not directly answer the question in their study. In addition, ER stress could be caused by solid phases in itself as shown in ref 19.

"The destabilization effect of Toc was stronger than that of Toc3, since the amount of Toc in the So phase was larger" and "It was also demonstrated that the amount of Toc in the So phase was larger than that of Toc3,". This is assumed from the DSC data and while they are likely conclusions from the data the wording is too strong, change to probably.

The authors write "Since the formation of phase-separated structures is influenced by lipid composition, the formation of lipids with low Tm, such as polyunsaturated lipids, may inhibit So phase formation in the ER membrane20"

Reference 20 refers mainly to ternary model membrane systems so is not so relevant for the formation of So in cholesterol-free membranes or ER. Increasing the unsaturation in the Low-Tm lipid in mixtures with disaturated PLs in binary model membranes has been shown to increase So thermostability see Biophys J. 2015 Nov 3; 109(9): 1907–1916. However, maybe an increased concentration of polyunsaturated FFA in the cell could compete with palmitate for the synthesis of phospholipids in the ER, as shown for the unsaturated oleic acid (OA) in J Lipid Res 47, 2726–2737 (2006) and for OA and DHA in ref 19? Therefore the effects in ER and model membranes may differ and these references could possibly clarify the authors' statement.

Author's Response to Peer Review Comments:

Dear Prof. Editor

Thank you for giving us the opportunity to submit a revised draft of our manuscript titled 『Suppression of Amyloid-β Adsorption on Endoplasmic Reticulum Stress-Mimicking Membranes by α-Tocopherol and α-Tocotrienol』 to 『The journal of physical chemistry letters』

We appreciate the time and effort that you and the reviewer have dedicated to providing your valuable feedback on our manuscript. We are grateful to the reviewer for the insightful comments.

Considering the reviewer's valuable comments, we have prepared revised version of our manuscript. We have incorporated changes to reflect most of the suggestions provided by the reviewer.

We have highlighted the changes within the revised version of our manuscript.

Here is a point-by-point response to the reviewer's comments and concerns.

I hope our responses to the reviewer are satisfactory and our revised version of the manuscript is suitable to be published in the journal of physical chemistry letters.

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Reviewer's Comment 1:

The authors' statement that ER forms the solid ordered phases should be rewritten as "may in special cases of ER stress form solid ordered phases".

The authors need to highlight that ER can form solid phases only in these special circumstances (excess palmitate or other saturated FFA feed to cells) even if it is highly relevant for biological systems.

It is also misleading to say that DOPC/DPPC membranes are "ER mimicking membranes", they should be stated as "ER stress mimicking membranes".
These points need to be considered and rewritten throughout the whole text.

Our Response 1:

Thank you very much for your valuable comment about solid phase formation of ER. Considering the reviewer's comment, we changed "ER-mimicking membrane" to "ER stress-mimicking membrane" throughout the manuscript including the title and added a description about S_o phase formation upon the addition of saturated lipids.

"ER-mimicking membrane"→"ER stress-mimicking membrane"

1. Title
2. Abstract
3. Keyword
4. p4
5. p5
6. p11
7. p12
8. p13

"Saturation of ER membrane lipids is known as ER stress[19,20]. When long-chain fatty acids, such as palmitate, are fed, saturated lipids are synthesized, forming S_o phase on ER membrane, leading to cell death[21]. Therefore, we considered that the formation of S_o phase is related to ER stress."

1. p4

Reviewer's Comment 2:

The authors have also used the fluorescent probe Rhodamine which is known to be prone to lipid oxidation. The authors do mention that the time exposure was limited, which is good.
Still, it would be good to confirm some of the results with another Ld-sensitive fluorophore probe, e.g., FAST DiO or DiD-C18.

Our Response 2:

Thank you for the comment about lipid oxidation. We have performed an additional experiment, as you suggested, to evaluate the effects of Rhodamine-DHPE oxidation on S_o/L_d phase separation.

As examples of other fluorophores, we used 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-DOPE) and 1-palmitoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl}-*sn*-glycero-3-phosphocholine (NBD-PC (18:1-06:0)) as fluorescent probes for L_d phase. These probes have been used to highlight the L_d phase in S_o/L_d[1]. Representative results shown below confirmed shrinkage and disappearance of S_o/L_d by addition of Toc and Toc3 (Fig. 1).

This result is basically the same as the case of Rhod-DHPE. Therefore, we concluded that use of Rhodamine-DHPE as a probe has no unexpected oxidation effect on phase separation.

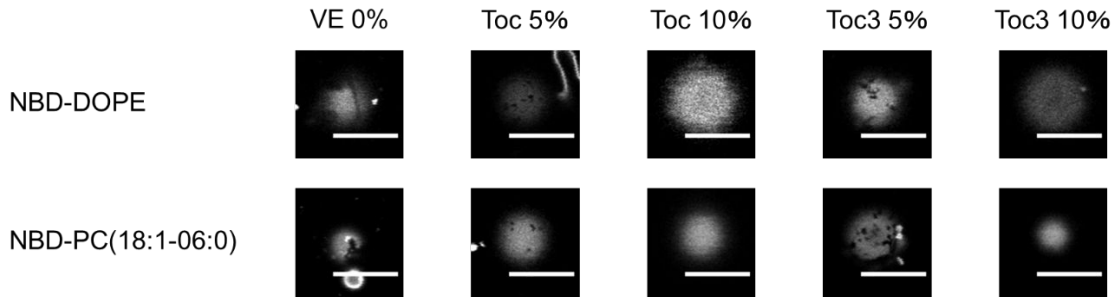

Fig. 1 Microscopic images obtained by confocal laser scanning microscopy

Liposomes were prepared with the following composition: DOPC/DPPC/VE = 50:50:0,

47.5:47.5:5, and 45:45:10. All liposomes contain 1 mol% fluorescent probe. Scale bars, 10μm

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Reviewer's Comment 3:

The sentence "We hypothesized that the accumulation of Aβ on the S_o phase causes ER stress." in the introduction, could be better placed in the discussion/conclusion part because while likely the authors do not directly answer the question in their study. In addition, ER stress could be caused by solid phases in itself as shown in ref 19.

Our Response 3:

Thank you for your suggestion for revision. It is certainly premature to state in the introduction that we have hypothesized about ER stress without performing any cellular experiments.

Following the reviewer's suggestion, we have moved the statement to the discussion/ conclusion part in the revised version (page 12 in the revised version).

1: p4

2: p12

Reviewer's Comment 4:

"The destabilization effect of Toc was stronger than that of Toc3, since the amount of Toc in the S_o phase was larger" and "It was also demonstrated that the amount of Toc in the S_o phase was larger than that of Toc3,".

This is assumed from the DSC data and while they are likely conclusions from the data the wording is too strong, change to probably.

Our Response 4:

Thank you very much for the comment.

We agree with the reviewer's comment.

Since we have not quantified the distribution of Toc and Toc3, it is indeed too strong to conclude that the amount of Toc in the S_o phase was larger than that of Toc3.

In the revised manuscript, we have inserted the 'probably' at appropriate positions.

1: p9

2: p13

Reviewer's Comment 5:

The authors write "Since the formation of phase-separated structures is influenced by lipid composition, the formation of lipids with low Tm, such as polyunsaturated lipids, may inhibit So phase formation in the ER membrane[20]"

Reference 20 refers mainly to ternary model membrane systems so is not so relevant for the formation of S_o in cholesterol-free membranes or ER.
Increasing the unsaturation in the Low-Tm lipid in mixtures with disaturated PLs in binary model membranes has been shown to increase So thermostability see Biophys J. 2015 Nov 3; 109(9): 1907–1916.
However, maybe an increased concentration of polyunsaturated FFA in the cell could compete with palmitate for the synthesis of phospholipids in the ER, as shown for the unsaturated oleic acid (OA) in J Lipid Res 47, 2726–2737 (2006) and for OA and DHA in ref 19?
Therefore, the effects in ER and model membranes may differ and these references could possibly clarify the authors' statement.

Our Response 5:

Thank you for pointing out very important issue.
Indeed, as you have stated, the presence of DHA in model membranes should be considered to contribute to S_o phase formation at lower concentrations of saturated lipids.
On the other hand, in cellular experiments, the addition of DHA inhibits the conversion of palmitic acid, which is important for So phase formation, into a membrane component, thus preventing the formation of the So phase.
Considering the reviewer's comment, in the revised manuscript, we changed the wording of the relevant part as follows:
" In ternary model membranes (DOPC, DPPC, and Chol), the disappearance of S_o/L_d phase separation occurs when the ratio of DOPC to DPPC increases[22]. However, increasing the unsaturation in the low-Tm lipid in mixtures with desaturated phospholipid in binary model membranes has been shown to increase S_o thermostability[44]. An increased concentration of polyunsaturated free fatty acids in the cell may compete with palmitate for the synthesis of phospholipids in the ER, as shown for the unsaturated oleic acid and DHA[21,45]. Therefore, the effects in ER and model membranes may differ."

1: p13

In addition to the comments received, minor corrections have been included.

Reference

(1) Weng, C.-J.; Wu, J.-P.; Kuo, M.-Y.; Hsueh, Y.-W. The Influence of NBD Fluorescent Probe on Model Membranes Containing POPC and DPPC. *Molecular Membrane Biology* **2016**, *33*, 23–28.