POLYMODAL DOSE - EFFECT OF ALPHA-TOCOPHEROL ON LIPID DINAMIC STRUCTURE OF CELL MEMBRANES *IN VITRO.*

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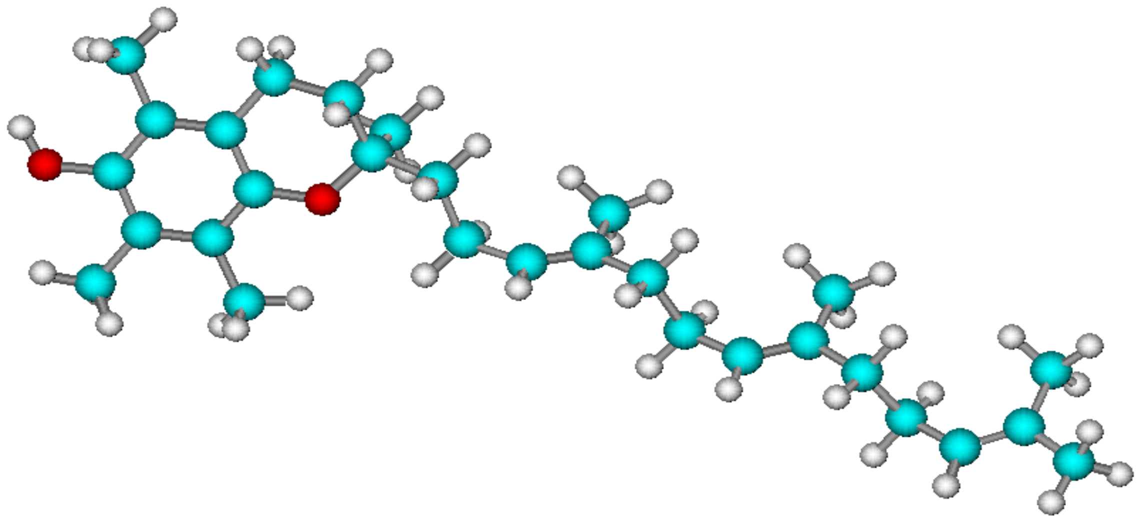
α-tocopherol (vitamin E)



The lipophilic α-tocopherol is localized in all biological membranes

- One of the most effective natural antioxidant

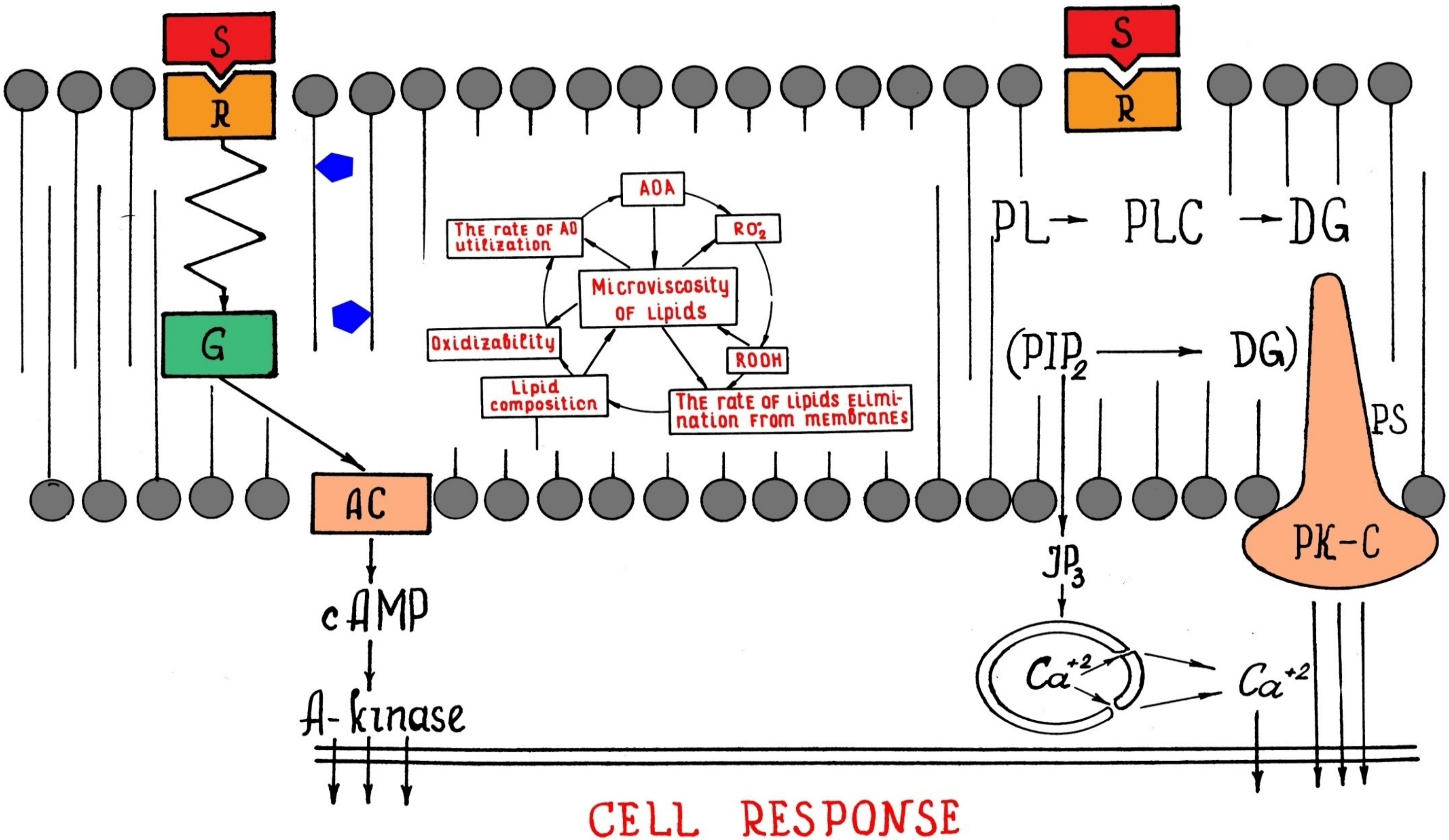
- Inhibitor of lipid peroxidation

- Structural factor in membrane lipids

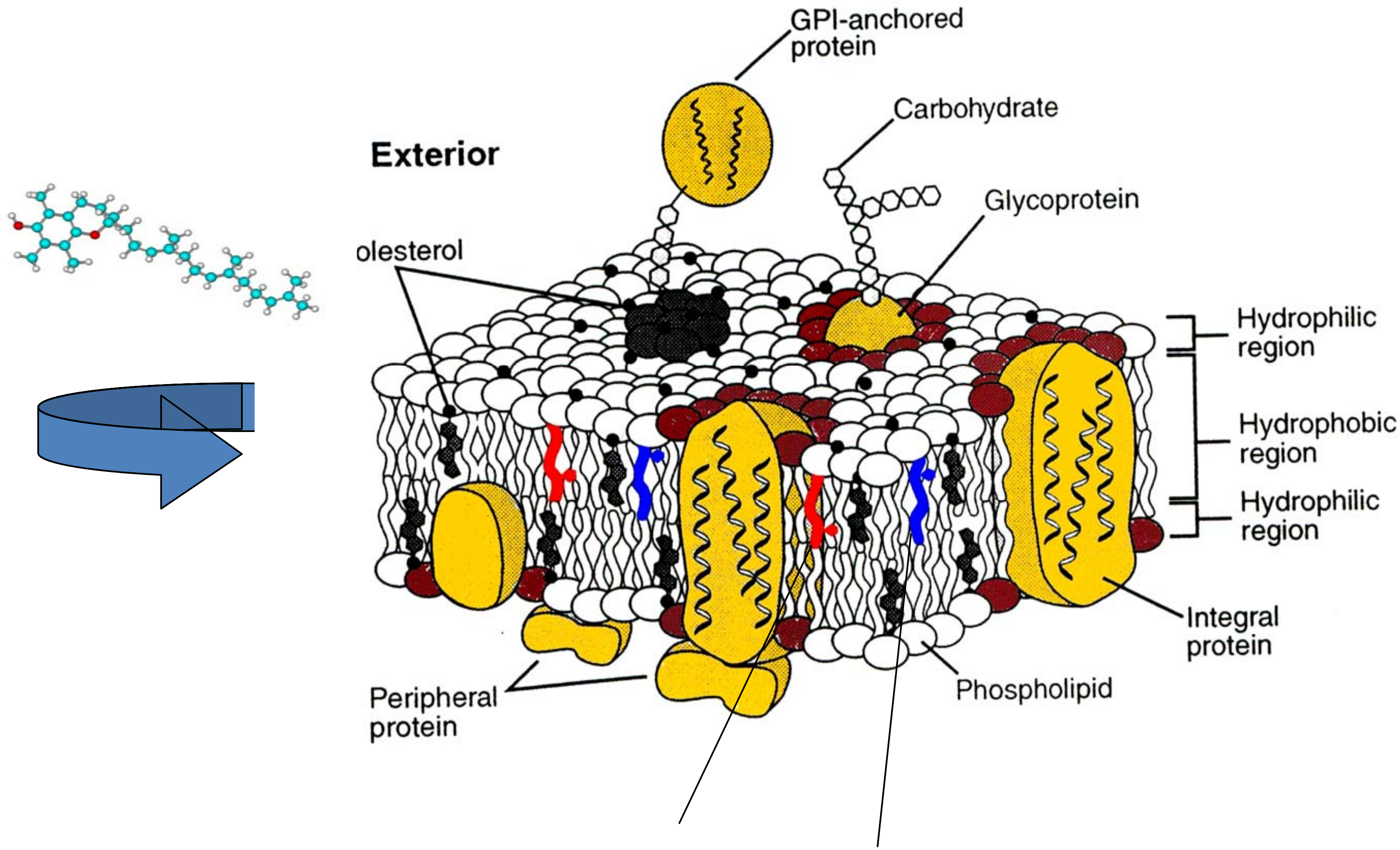
- α-tocopherol forms the domains with phospholipids,

- reacts with the products of lipid hydrolysis and prevents a destruction of cell membranes

THE SYSTEMS OF SECOND MESSENGERS AND LIPID PEROXIDATION IN PLASMATIC MEMBRANE



The aim of this work was to study the effect of α-tocopherol in a wide range of concentration (10^{-3} -10^{-23} M) on the dynamic lipid structure of cell membranes *in vitro*



Ethanol-water solutions of α-TL were obtained by method of consecutive dilutions by next nearest order of its initial 10^{-1} M solution with ethanol (high rectification) to the concentration of 10^{-3} M, and then with bi-distilled water to 10^{-23} M.

The structural dynamic state of membrane lipids was studied by EPR-method (spectrometer Bruker-EMX) using two spin-probes:
5-doxylstearic acid (5-DSA) is localized in the surface membrane lipids at ~8A^0
16-doxylstearic acids (16-DSA) is localized in the deep-lying hydrophobic regions at ~20 A^0 of membrane lipids.

Microviscosity value of the deep-lying hydrophobic lipid regions was estimated by a rotation correlation time (τ_c) of 16-DSA.

$$\text{Effect} = (\tau_{test} - \tau_{control}) / \tau_{control} \times 100\%$$

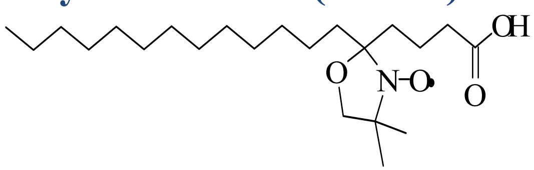
Rigidity of surface membrane lipids was estimated by order parameter (S) depending on amplitude of deviation a large axis of the ellipsoid of rotation spin-probe 5-DSA.

$$\text{Effect} = (S_{test} - S_{control}) / S_{control} \times 100\%$$

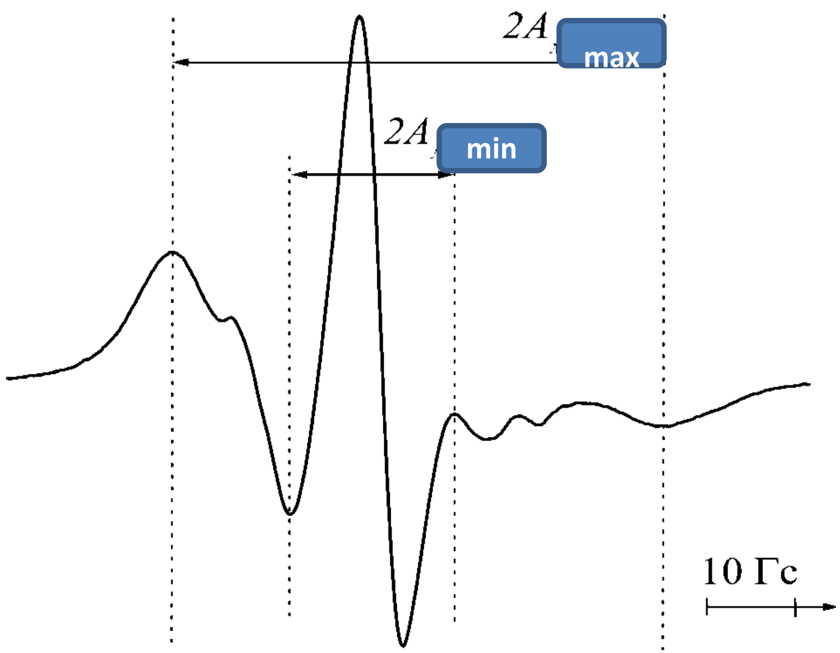
Relative standard errors for these effects were obtained after statistical treatment of all results by methods for parametric and non-parametric statistics with the use of computer program packages Statistica and Origin 6.1 at statistical reliability of 95%.

Typical EPR-spectra of spin probes in cell membranes

5-doxylstearic acid (5-DSA)



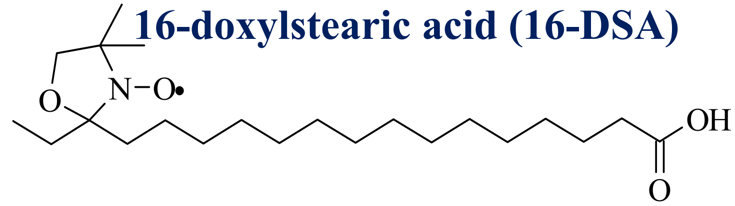
~8A^0



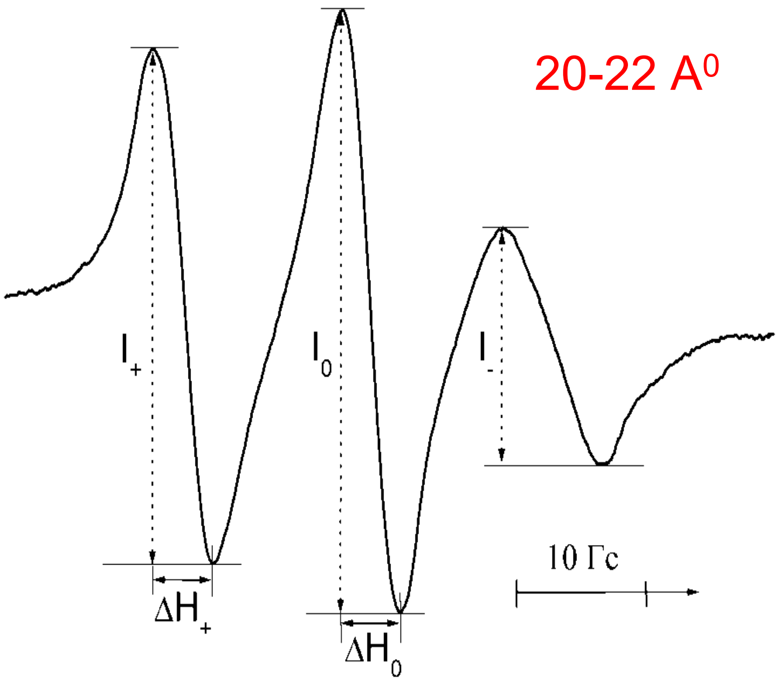
$$S = 1,66 \cdot \frac{A_{max} - A_{min}}{A_{max} + 2A_{min}}$$

an order parameter

16-doxylstearic acid (16-DSA)



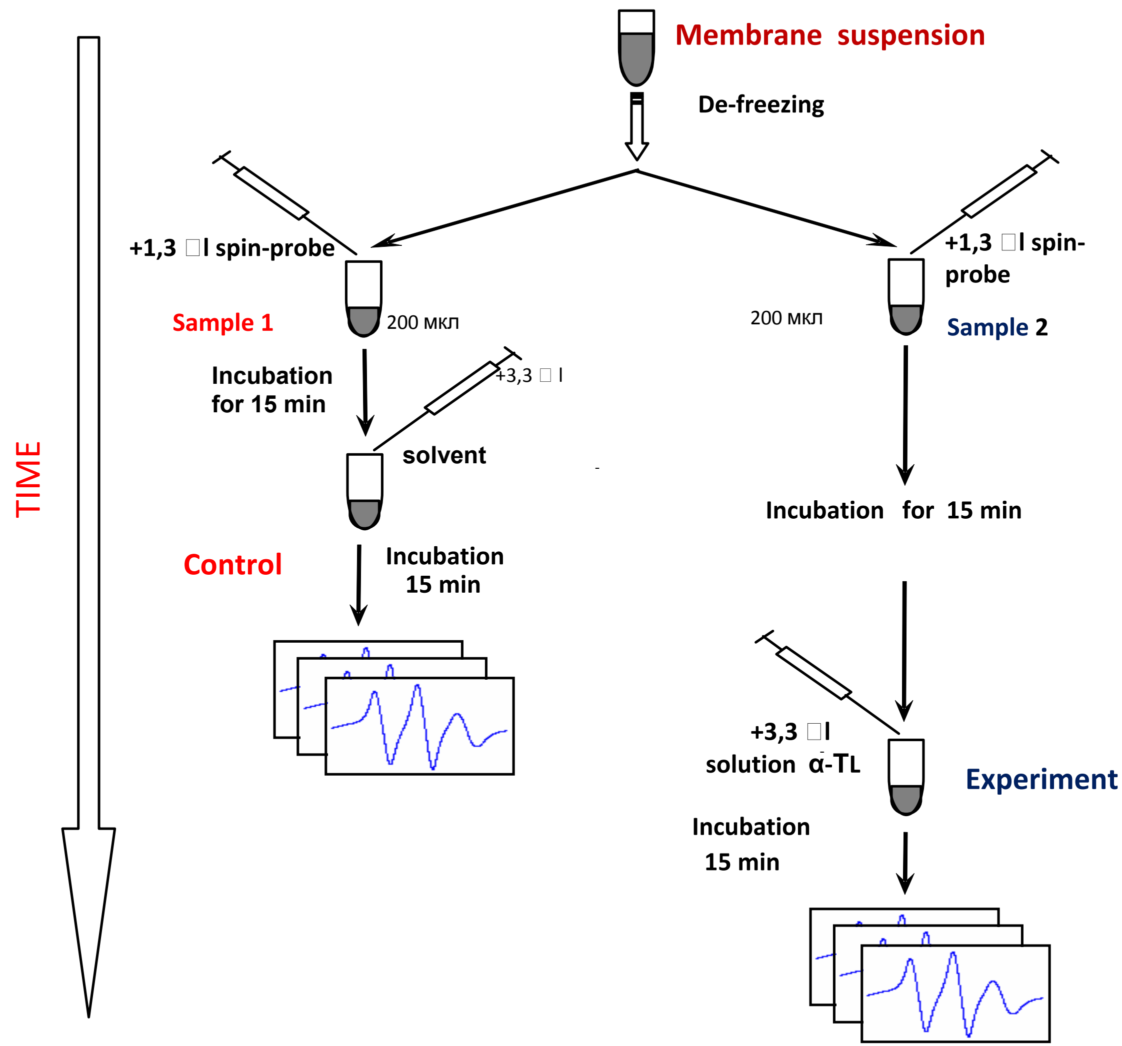
20-22 A^0



$$\tau_c = 6,65 \cdot \Delta H_0 \left(\sqrt{\frac{I+}{I_-}} - 1\right) \cdot 10^{-10}, s$$

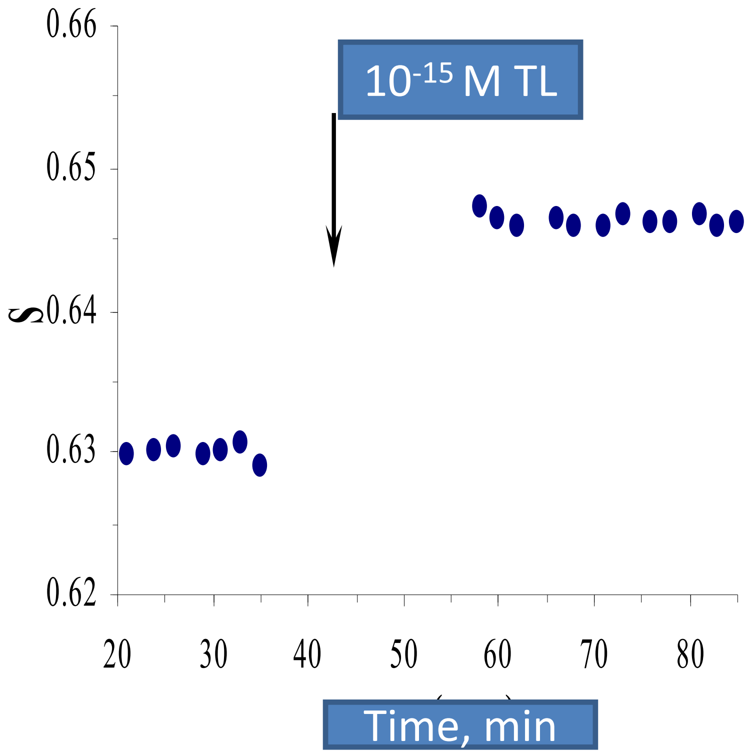
a rotational correlation time

The scheme of the experiments with cell membranes

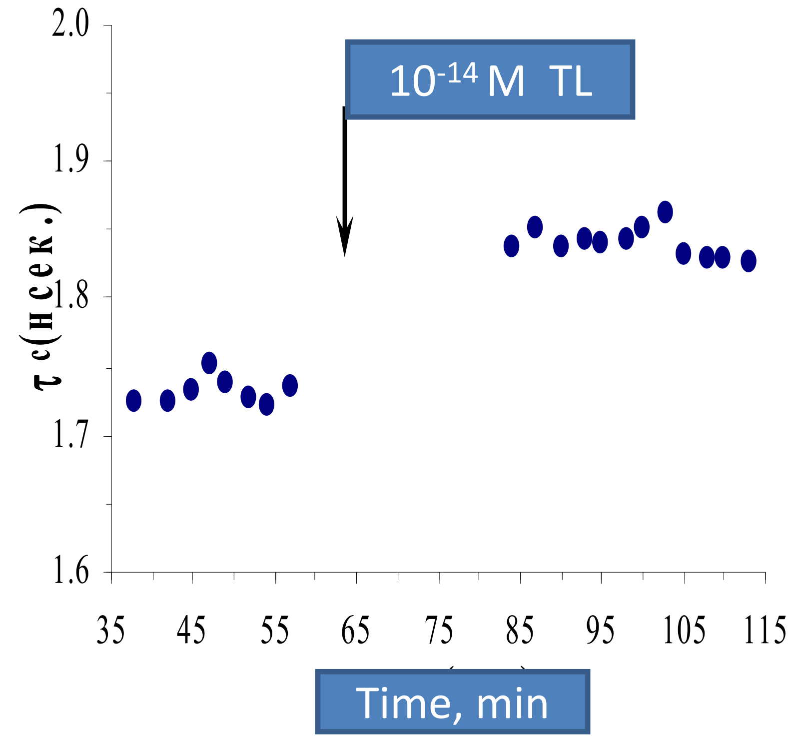


The typical experiments

endoplasmic reticulum membranes

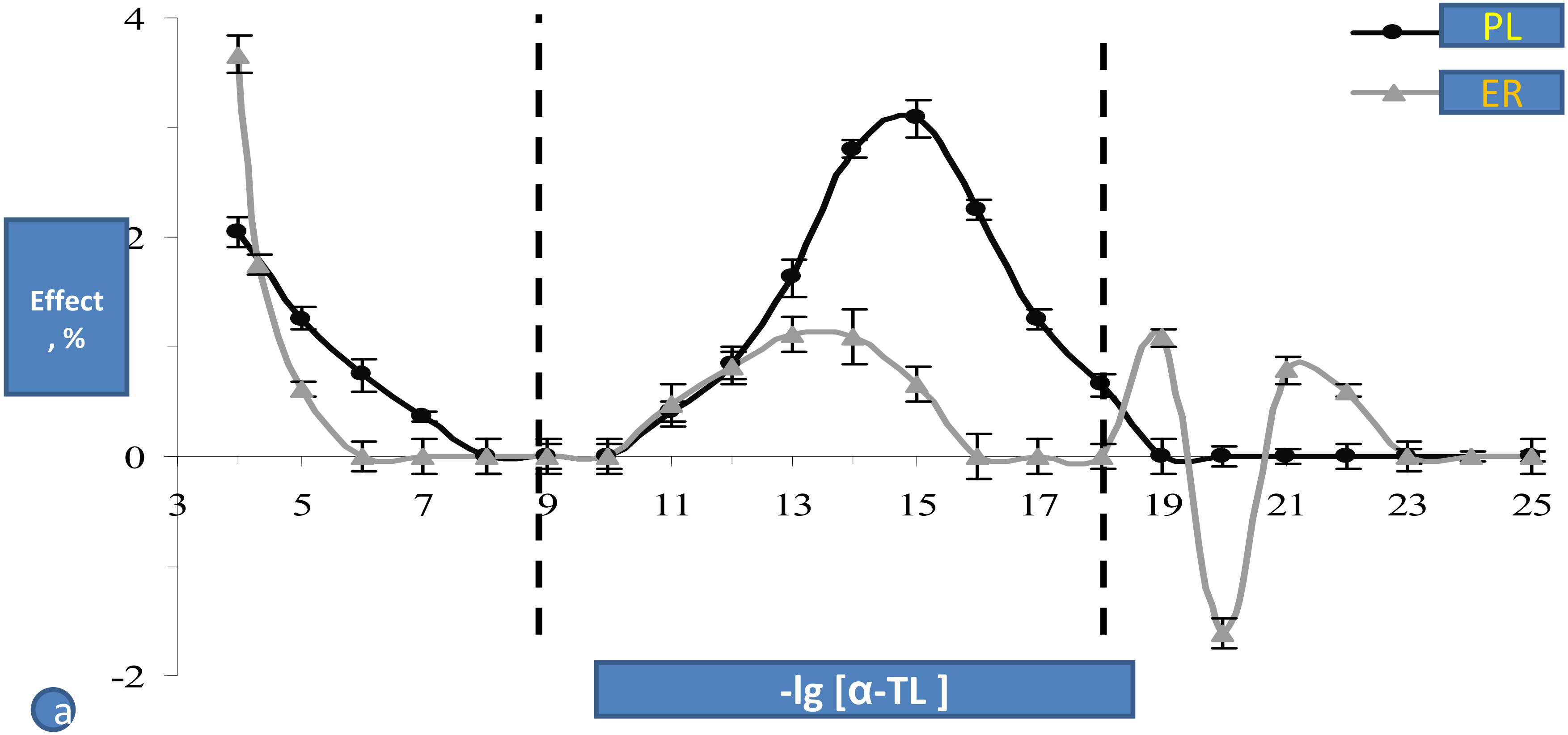


plasmatic membranes



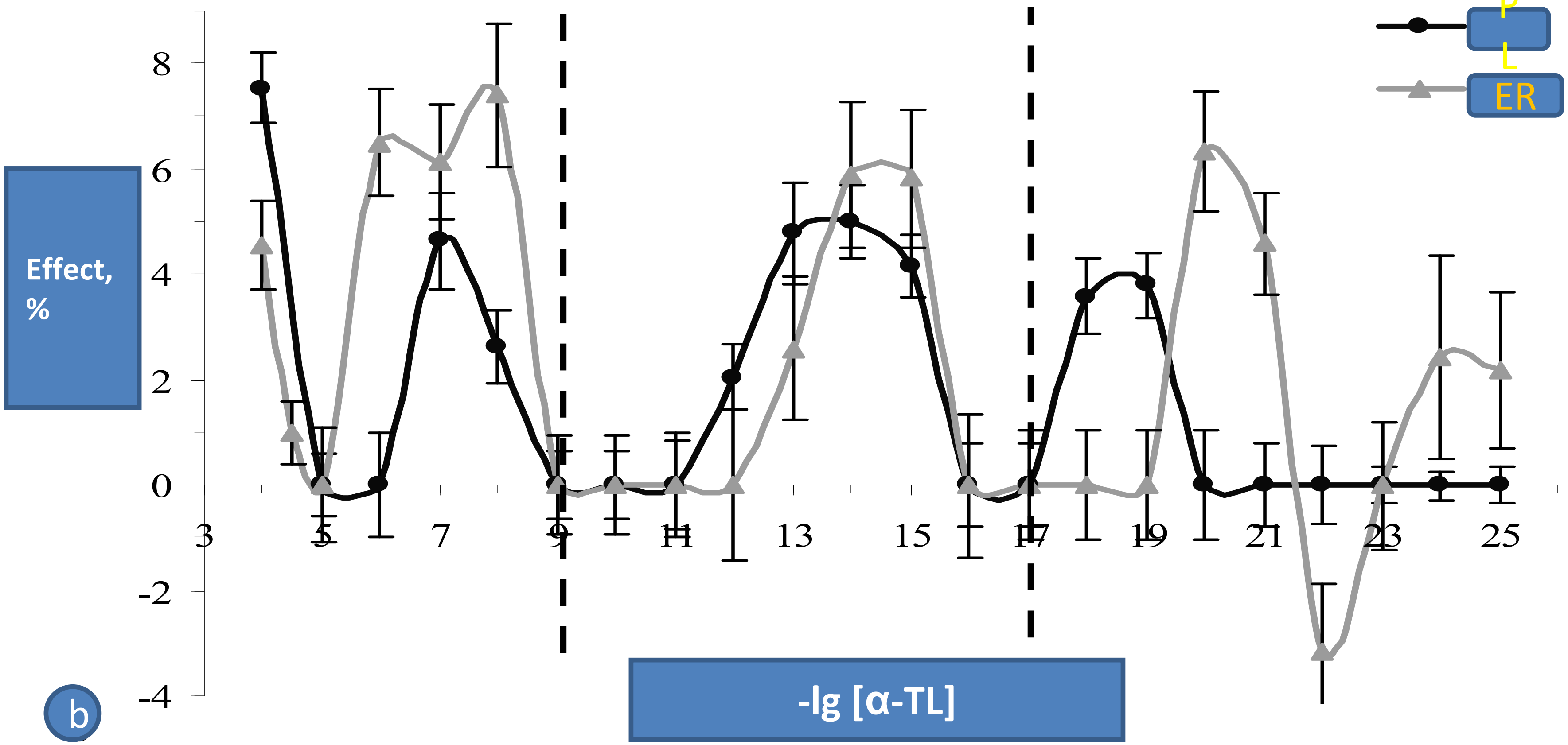
The changes of order parameter S spin-probe 5-DSA and a rotation correlation time - τ_c spin probe 16-DSA upon the action of α-tocopherol (TL) at the concentration 10^{-16} and 10^{-14} M correspondingly.
The concentration of protein in membrane suspension – 3 mg/ml.
The concentration of 16-DSA- $2*10^{-4}$ M. The temperature 293 ^{0}K.

The effect of α-tocopherol on the rigidity of surface lipids of membranes



The changes of order parameter ($S-S_0/S \times 100\%$) of 5-DSA in membane lipids depending on the concentration of α-tocopherol

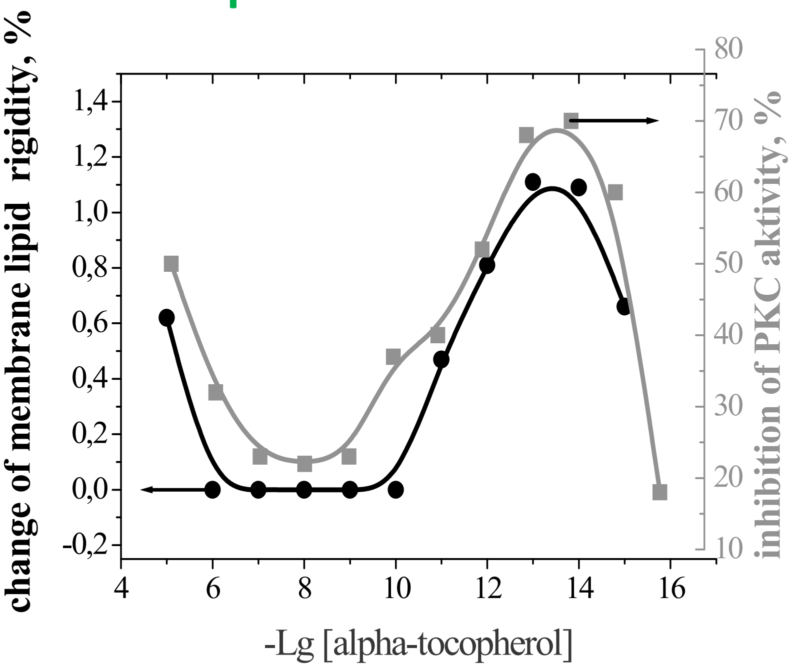
The effect of α-tocopherol on the microviscosity of deep-lying hydrophobic lipid regions of membranes



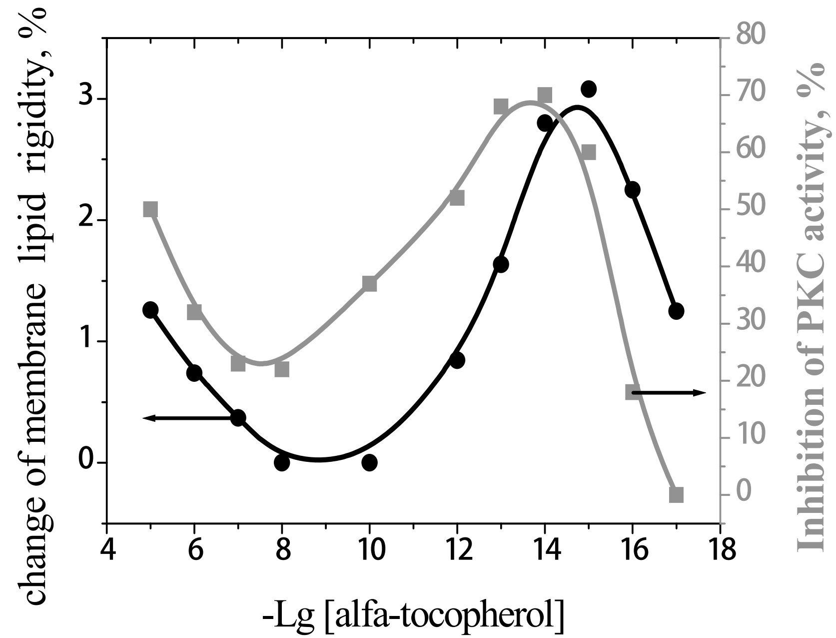
The changes of rotation correlation time (□c-□0/ □0 x 100%) of 16-DSA

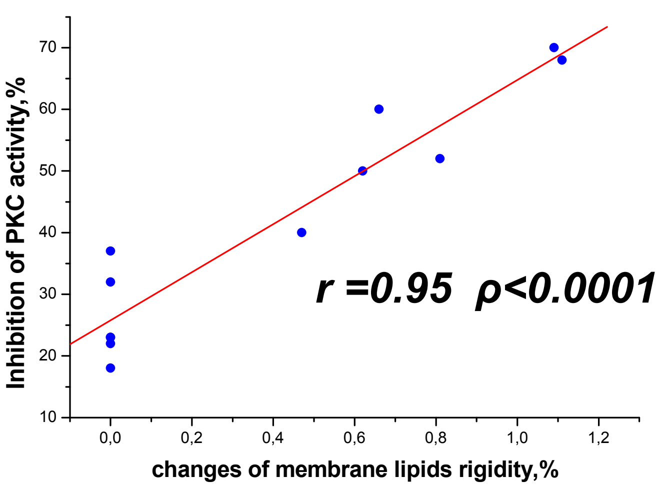
The relationship between the change of PKC activity and rigidity of surface lipids of membranes

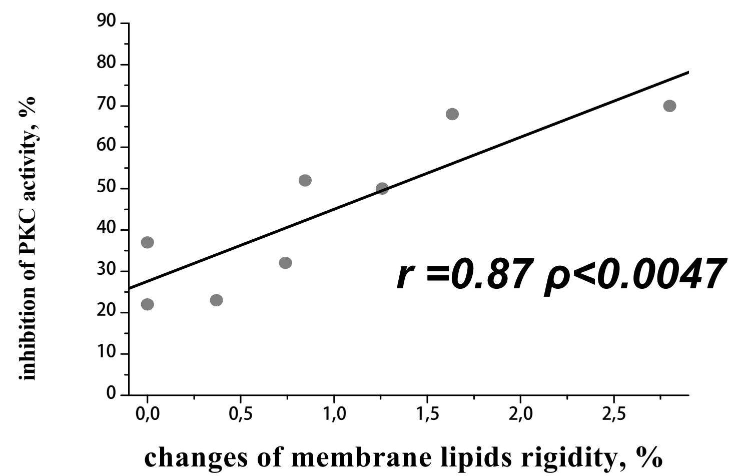
endoplasmic reticulum



plasmatic membranes

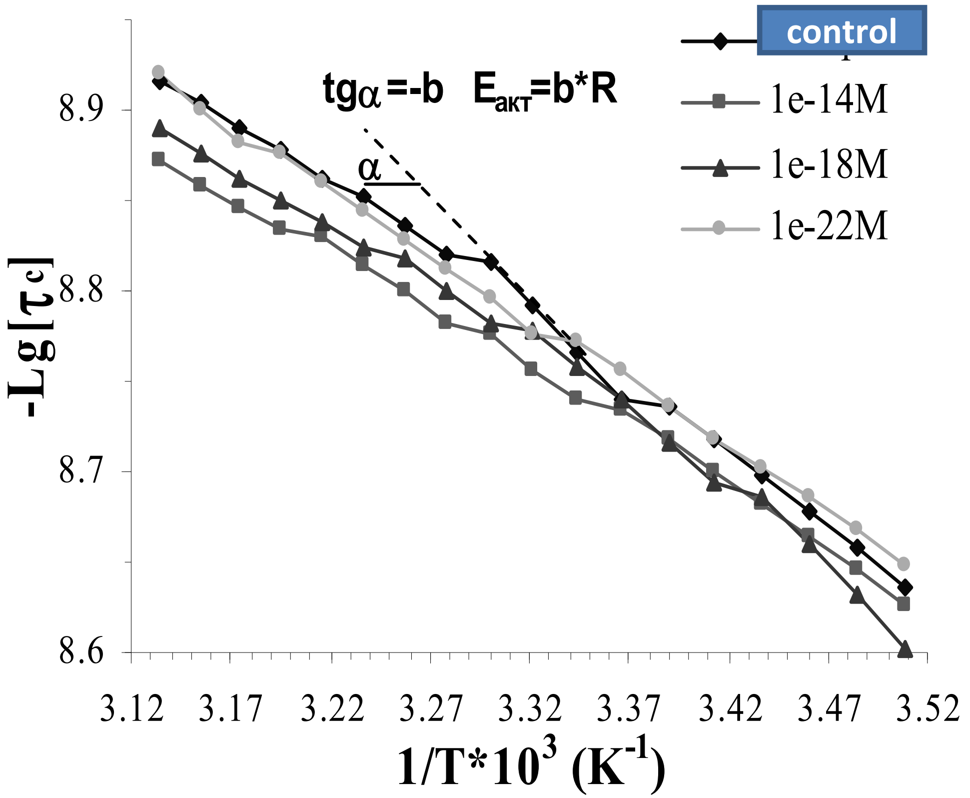




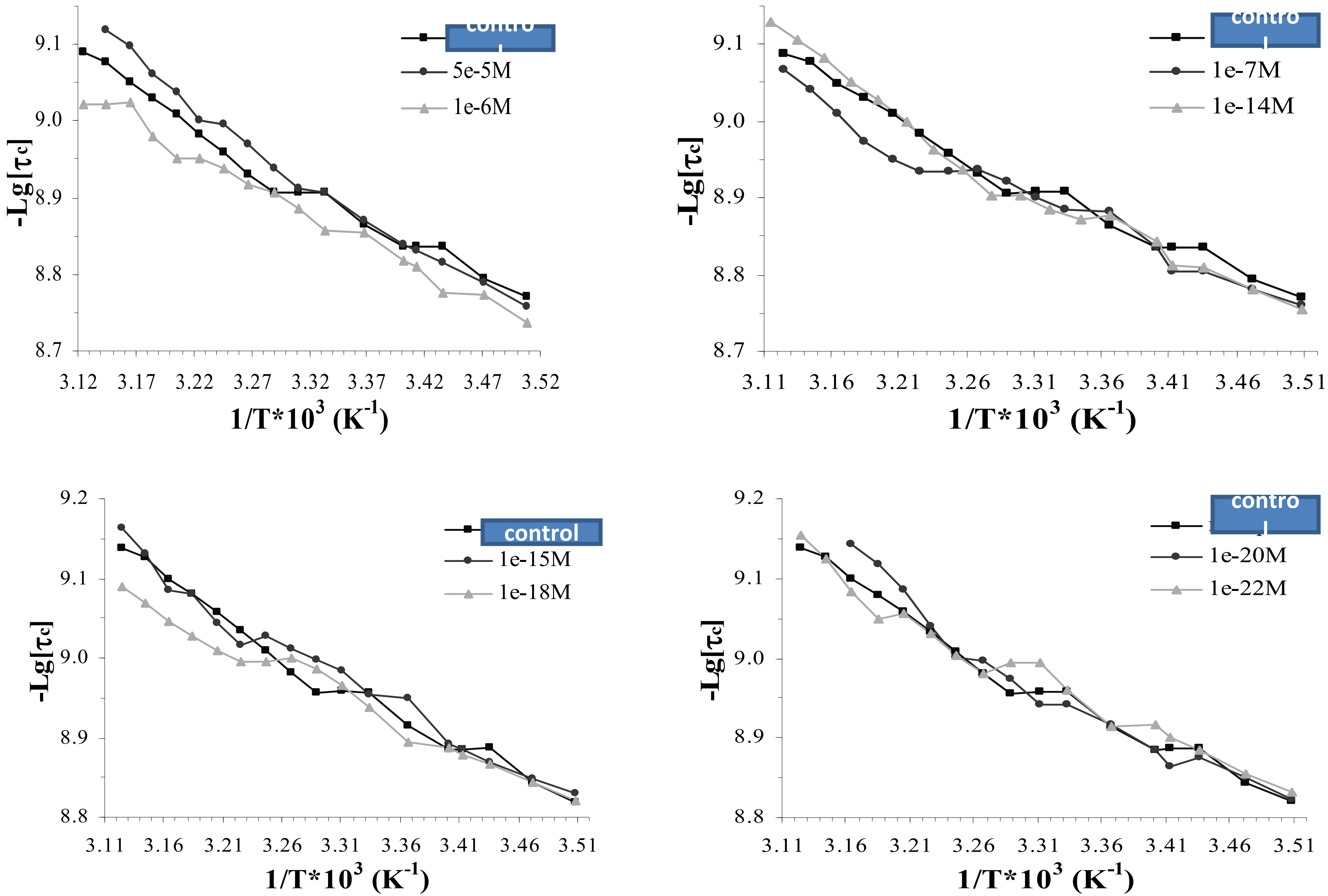


The important characteristics of structural-dynamic state of membrane are the quantity and quality of thermo-induced structural transitions (TST).

TST represent a cooperative transformation of microdomans of lipids upon raising the temperature, which are accompanied by jump-like change of the structural parameter of lipid bilayer.

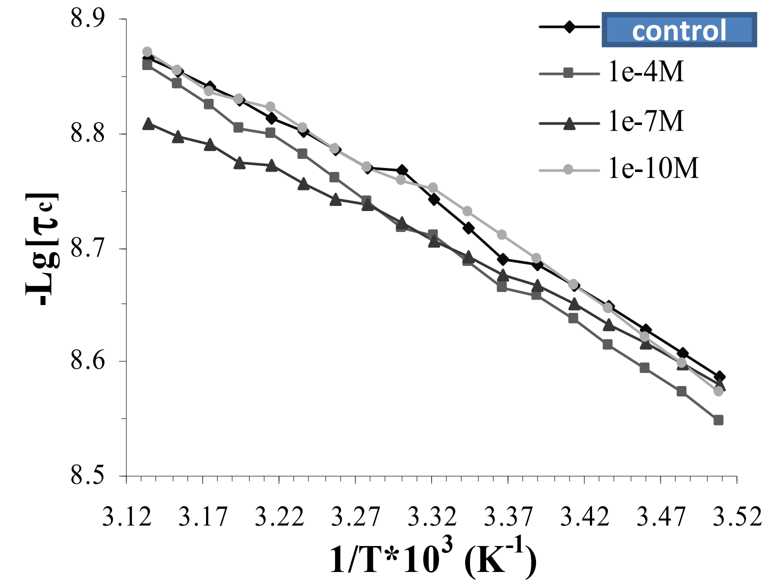


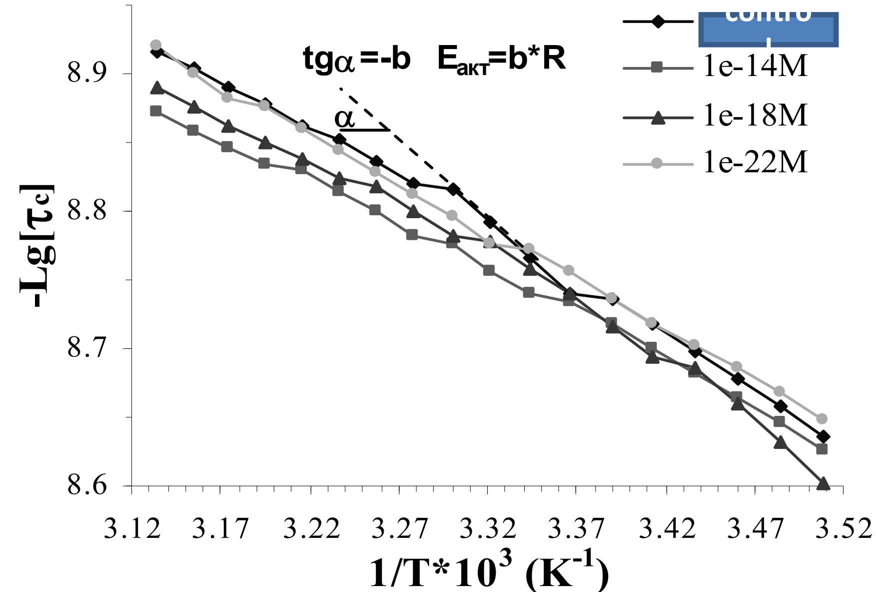
Temperature dependences of rotation correlation time (□c) 16-DSA presented in Arrhenius coordinates in hydrophobic lipid regions of ER- membranes

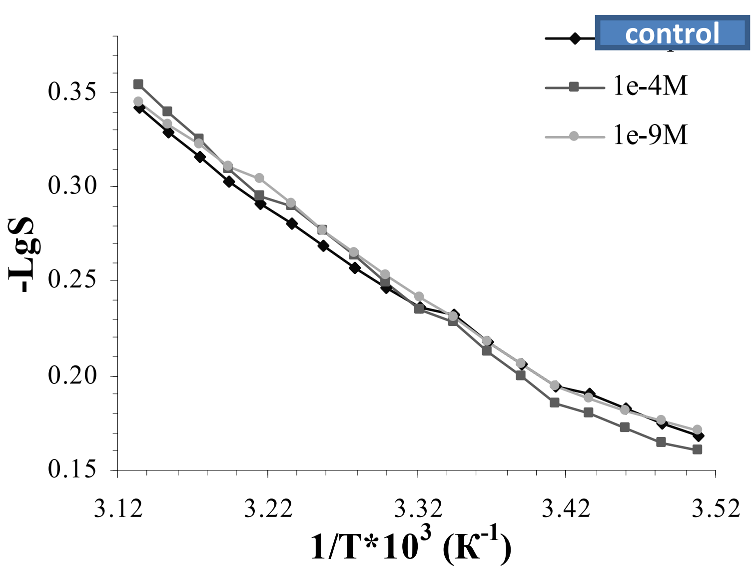


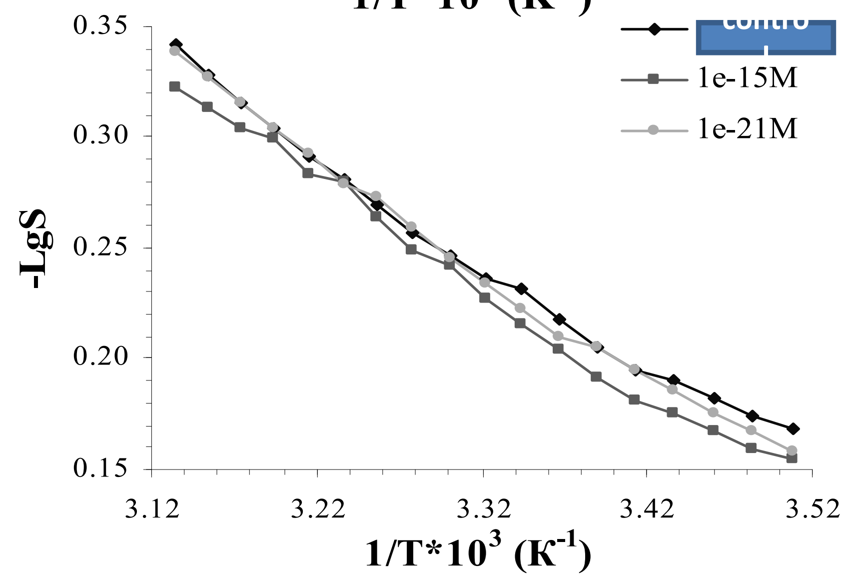
16-DSA

Temperature dependences of rotation correlation time (□c) 16-DSA and order parameter (S) 5-DSA presented in Arrhenius coordinates in hydrophobic and surface lipid regions of plasmatic membranes









The effect of different concentration of α-TL on the termoinduced structural transition in the surface lipid regions of membranes

endoplasmic reticulum

T,K	contro	10^{-4}M	10^{-8}M	10^{-13}M	10^{-17}M	10^{-19}M	10^{-20}M
285							
287							
289							
291							
293							
295							
296							
297							
299							
301							
303							
305							
307							
309							
311							
313							
315							
317							
319							

plasmatic membranes

T,K	control	10^{-4}M	10^{-9}M	10^{-15}M	10^{-21}M
285					
287					
289					
291					
293					
295					
297					
299					
301					
303					
305					
307					
309					
311					
312					
313					
315					
317					
319					

TST are appeared into the interval of physiologycal temperatures

The effect of different concentration of α-TL on the termoincuced structural transitions and their effective energy of activation E^{eff}_{act} (кJ/mol) in the deep hydrophobic lipid regions of membranes

endoplasmic reticulum

<table>
<tr><th>T,K</th><th>control</th><th>5*10^{-5}M</th><th>10^{-6}M</th><th>10^{-7}M</th><th>10^{-14}M</th><th>10^{-15}M</th><th>10^{-18}M</th><th>10^{-20}M</th><th>10^{-22}M</th></tr>
<tr><td>285</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>288</td><td></td><td></td><td rowspan="2">8±1.2</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>291</td><td rowspan="3">7,7±1.1</td><td></td><td rowspan="2">5,1±0.5</td><td rowspan="2">6,1±0.6</td><td rowspan="2">4,6±0.5</td><td></td><td rowspan="2">5,8±0.5</td><td></td></tr>
<tr><td>293</td><td></td><td></td><td></td><td></td></tr>
<tr><td>294</td><td></td><td></td><td></td><td></td><td></td><td rowspan="2">5,1±0.4</td><td></td><td rowspan="2">6,5±0.4</td></tr>
<tr><td>297</td><td></td><td></td><td rowspan="3">9,3±0.9</td><td rowspan="3">13,1±1.9</td><td rowspan="2">10,9±1.6</td><td rowspan="3">15,6±1.5</td><td rowspan="3">7,5±0.6</td></tr>
<tr><td>299</td><td></td><td></td><td></td><td></td></tr>
<tr><td>300</td><td rowspan="4">8,8±0.9</td><td rowspan="2">6,9±0.1</td><td></td><td></td><td></td></tr>
<tr><td>302</td><td></td><td></td><td></td><td></td><td></td><td></td><td rowspan="5">11,7±1.1</td></tr>
<tr><td>303</td><td></td><td></td><td></td><td rowspan="3">6,1±0.6</td><td></td><td></td><td></td></tr>
<tr><td>304</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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<tr><td>306</td><td></td><td></td><td></td><td rowspan="3">6,8±0.5</td><td></td><td></td><td rowspan="3">9,2±0.5</td><td rowspan="2">6,8±0.6</td></tr>
<tr><td>308</td><td></td><td rowspan="2">11±0.8</td><td></td><td></td><td rowspan="2">6,8±0.4</td><td></td></tr>
<tr><td>310</td><td></td><td rowspan="2">7,1±0.2</td><td></td><td></td><td></td></tr>
<tr><td>312</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td rowspan="2">9,8±0.7</td></tr>
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<tr><td>316</td><td></td><td></td><td rowspan="3">14,7±1.4</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>318</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>320</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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plasmatic membranes

<table>
<tr><th>T,K</th><th>control</th><th>10^{-4}M</th><th>10^{-7}M</th><th>10^{-10}M</th><th>10^{-14}M</th><th>10^{-18}M</th><th>10^{-22}M</th></tr>
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<tr><td>291</td><td></td><td></td><td></td><td></td><td></td><td rowspan="2">9.2±0.6</td><td></td></tr>
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<tr><td>295</td><td rowspan="2">6.5±0.3</td><td rowspan="2">7.6±0.4</td><td rowspan="2">6.2±0.2</td><td></td><td></td><td></td><td></td></tr>
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<tr><td>301</td><td></td><td rowspan="2">8.4±0.9</td><td></td><td rowspan="3">7.3±0.2</td><td></td><td rowspan="2">7.2±0.4</td></tr>
<tr><td>303</td><td rowspan="2">8.8±0.6</td><td></td><td rowspan="2">7.0±0.8</td><td></td></tr>
<tr><td>305</td><td></td><td rowspan="2">5.8±0.5</td><td></td><td></td></tr>
<tr><td>307</td><td></td><td></td><td></td><td></td><td rowspan="2">6.7±0.7</td><td></td></tr>
<tr><td>309</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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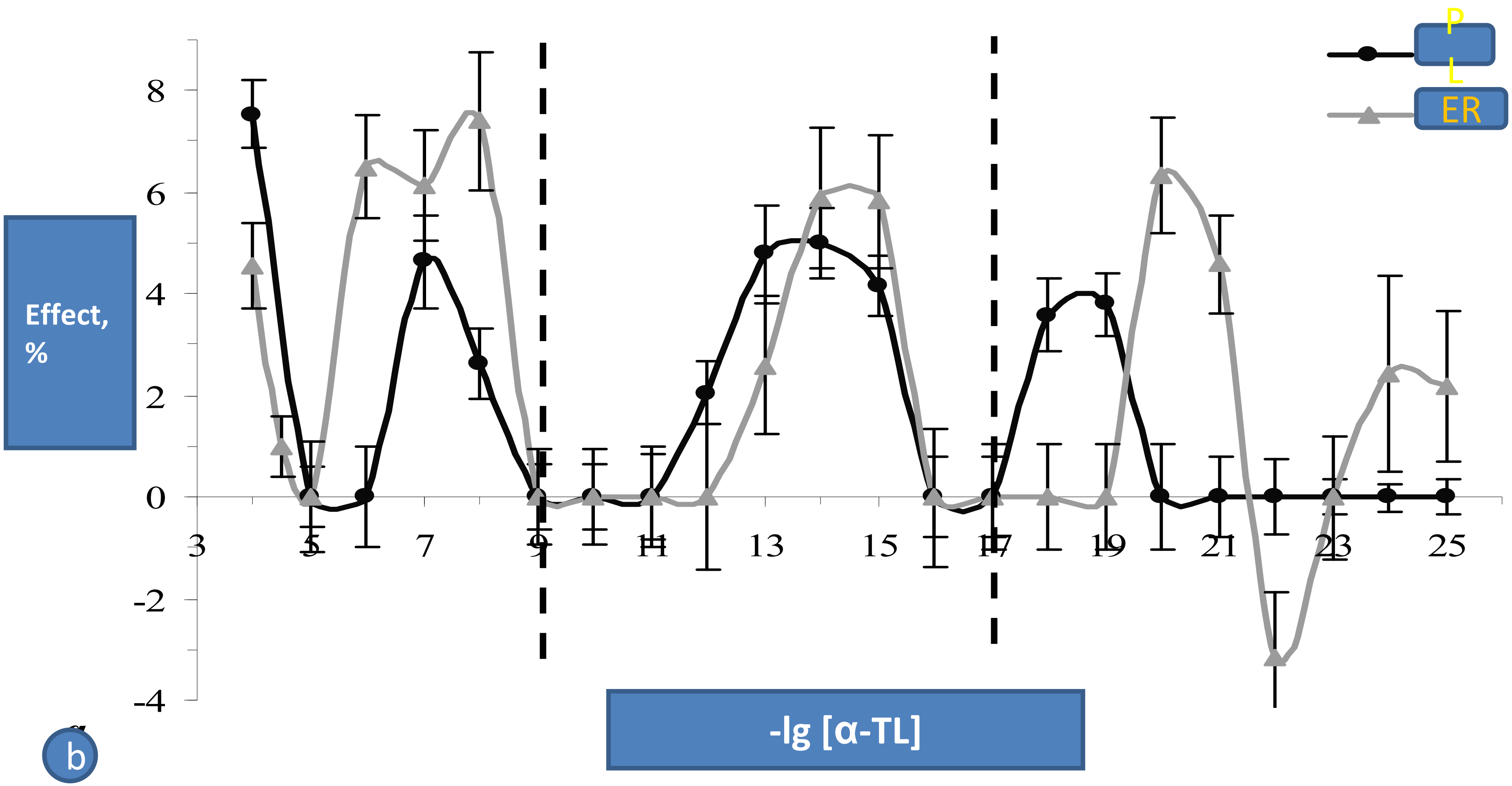
TST curves are appeared in the range of physiological temperatures corresponding to maximum and minimum of “dose-effect” curves.

The effect of α-tocopherol on the microviscosity of deep-lying hydrophobic lipid regions of membranes

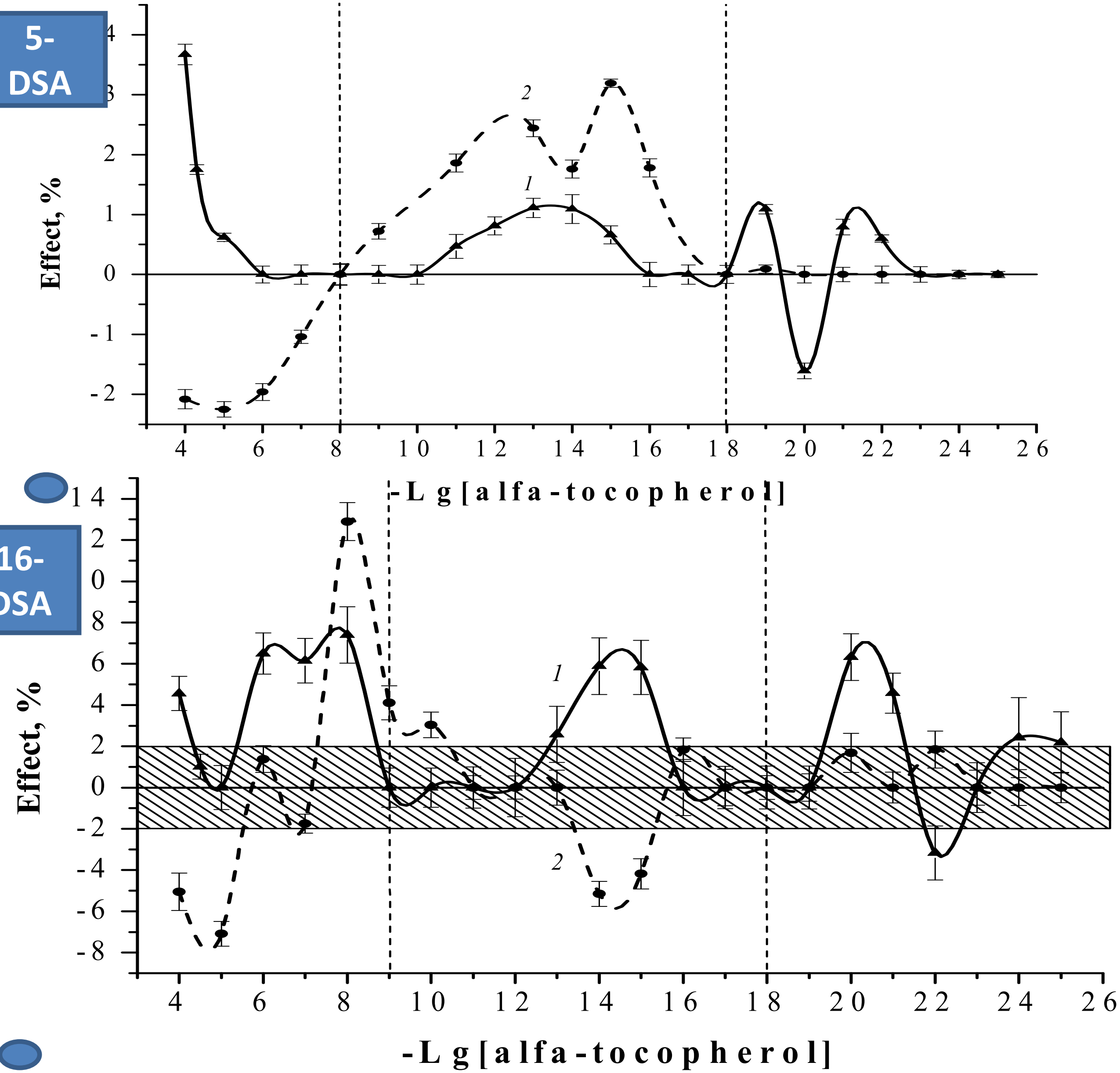


The changes of rotation correlation time (□c-□0/ □0 x 100%) of 16-DSA

Different effect of α-tocopherol dissolved in polar and nonpolar solvents.

5-DSA

16-DSA



1 - Polar (water-ethanol) solvent

2. Nonpolar (petrolatum oil) solvent

Three “waves” of change of lipid dymamic state are obtained in the membranes under the effect of α-tocopherol

1. **The range of physiological concentrations (10^{-4}M-10^{-9}M)** – a restriction of conformational mobility of lipids as a result of α-tocopherol incorporation into the membranes;
2. **The interval of low and ultra low doses (10^{-9}M-10^{-17}M)** – a specific interaction with binding sites on the membrane: protein kinase C and formation of lipid micro-domains induced by α-tocopherol in the membrane (indirect evidence is appearance of additional termoinduced transitions at physiological temperature);
3. **The area of “apparent” concentrations ($<10^{-17}$M)** – solvent polarity plays a key role in the mechanism of action of α-TL.

The mechanism of α-tocopherol effect?

It was shown (group of acad. Konovalov):

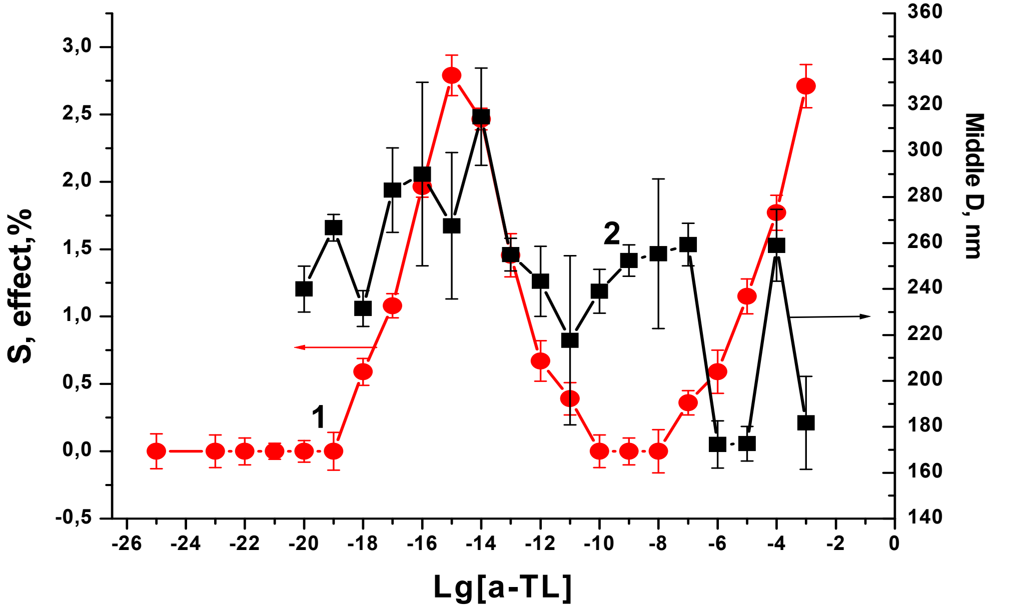
an spontaneus formation of charged nanoassociates in water-solutions prepared by consecutive dilutions;

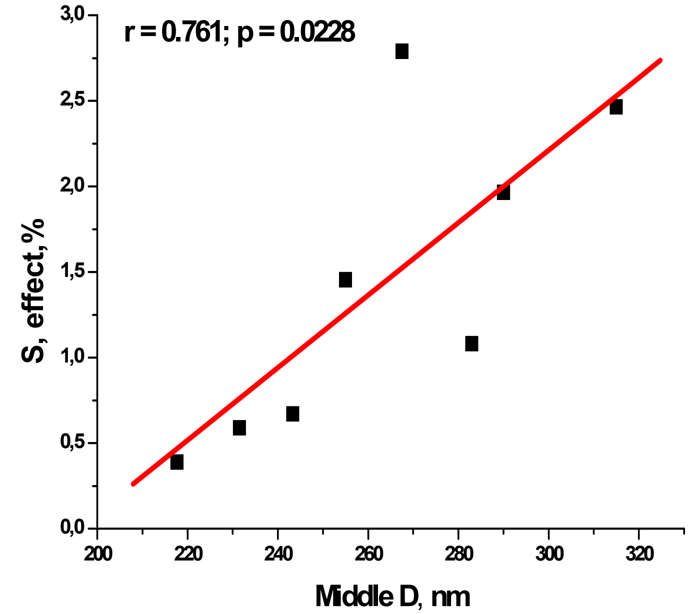
nanoassociates consist of hydro-ions or molecules of substances and ordered water structure:

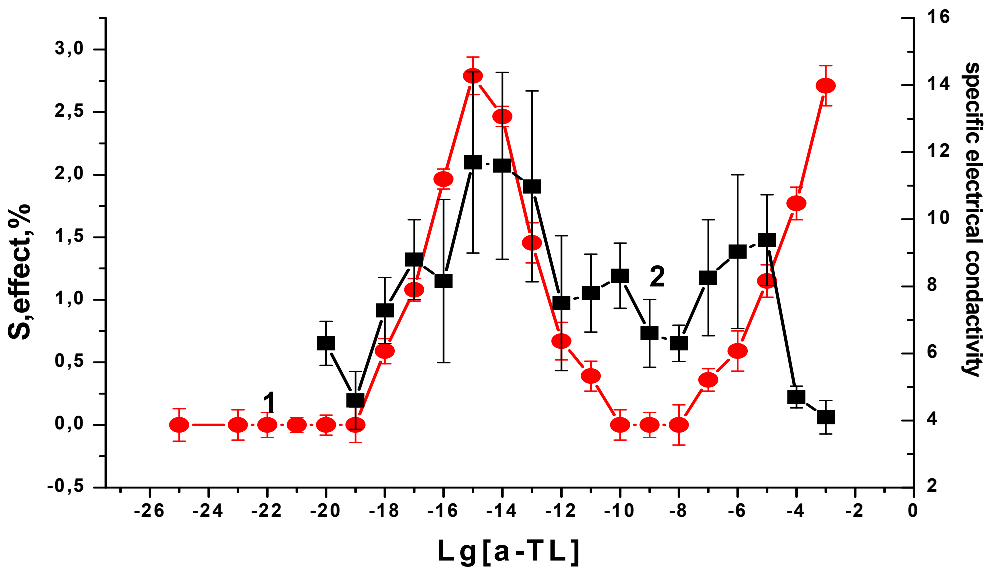
- **effective hydrodynamic diameter (D) 100-300 nm,**
- **ζ-potencial -2 - 20 мV;**
- **dielectric penetration (□)**

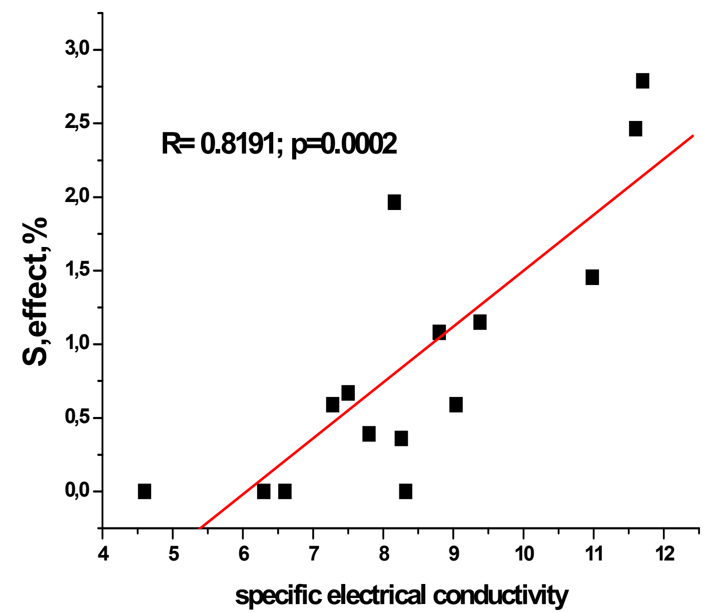
changes of physico-chemical properties of water-solutions unlinear depend on the concentration of substances

The correlation between the changes of parameters S of PL lipids, diameter of nanoaccosiates and specific electrical conductivity of α-TL-solutions.









Conclusions

Polymodal effect of α-tocopherol in a wide range of concentrations on the dynamic lipid structure of cell membranes is typical to action of biological active substances at ultra-low concentrations.

The increase of rigidity of PL and ER membranes correlates with an inhibition of protein kinase C activity.

A possible mechanism of α-TL effect can be related with a formation of nanoassociates and the changes of physico-chemical properties of α-TL solutions.

Thank You for Your attention!