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The influence of selected amino acids on the dynamic properties of the liposome membranes: ESR study

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Abstract. In this work the changes in the fluidity of liposome membranes caused by alanine and butyrine derivatives (Ac-Ala-NMe_2 and Ac-Abu-NMe_2) were investigated. Liposomes were obtained in the process of egg yolk lecithin (EYL) sonication. The concentration of the admixture in the proportion to EYL varied from 0 to 25% mole. The electron spin resonance (ESR) spectroscopy was used with two different spins probes. Each spin probe penetrates different regions of liposome membrane. The TEMPO probe occurs both in the hydrophobic part of the membrane and in the water environment what allows to determine the spectroscopic parameter *F* of division of this probe into the membrane and its water surrounding. DOXYL is localized in the central part of the lipid bilayer and is used to obtain the spectroscopic parameter τ – rotation correlation time – whose value gives information about fluidity changes in the middle of the lipid bilayer. The study indicated that the tested as admixtures N-methylated model peptides significantly changed the fluidity of liposome membranes. The dynamic of this process depends both on amino acids derivative and on the membrane region. Both studied compounds increased the fluidity of the surface layer of liposome membrane. At the same time, butyrine derivative caused the stiffening of the middle part of liposome bilayer, but alanine derivative slightly increased the fluidity of this region.

Key words: EYL liposomes • electron spin resonance (ESR) method • N-methylation • amino acids • peptides

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Introduction

Amino acids are bipolar compounds with carboxylic and amine ends. The former one undergoes deprotonation in basic solution while the latter one is protonated in acidic media. Thus, neutral amino acids and zwiterions are present in the aqueous environment. Besides, they interact strongly with charged parts of other molecules and form strong intermolecular H-bonds. The association and interaction with solvent and other molecules is often studied using spectroscopic methods, including Fourier-transform nuclear magnetic resonance – FTIR/Raman, nuclear magnetic resonance (NMR) and electron spin resonance (ESR) [1–3]. The latter technique is very sensitive and enables obtaining both structural and dynamic information of a probe containing an unpaired electron spin. N-Alkylation of biologically active peptides can result in analogues with improved pharmacological properties, such as resistance to enzymatic degradation, receptor selectivity, enhanced potency and bioavailability [4]. Despite the excessive interest in the biological activity of peptides containing N-methyl amino acids, the consequences of the N-substitution for peptide-membrane interactions have not been thoroughly examined so far.

The aim of our current study was the determination of physical processes in a model liposome membrane

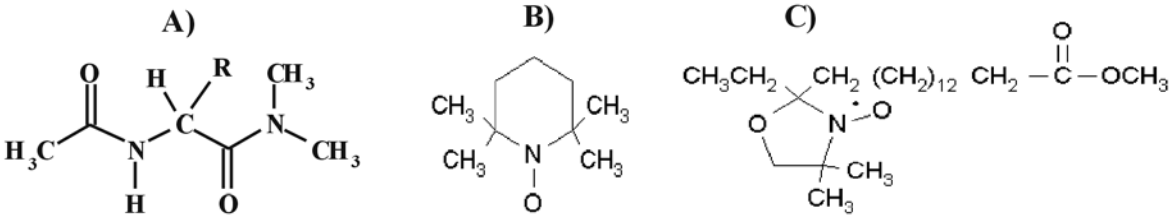

Fig. 1. General formula for the studied compounds: A) R=CH_3 for Ac-Ala-NMe_2, R=C_2H_5 for Ac-Abu-NMe_2; B) spin probe TEMPO: 2,2,6,6-tetramethyl-1-piperidinyloxy; C) spin probe 16-DOXYL methyl ester: 2-ethyl-2-(15-methoxy-15--oxopentadecyl)-4,4-dimethyl-3-oxazolidinyloxy.

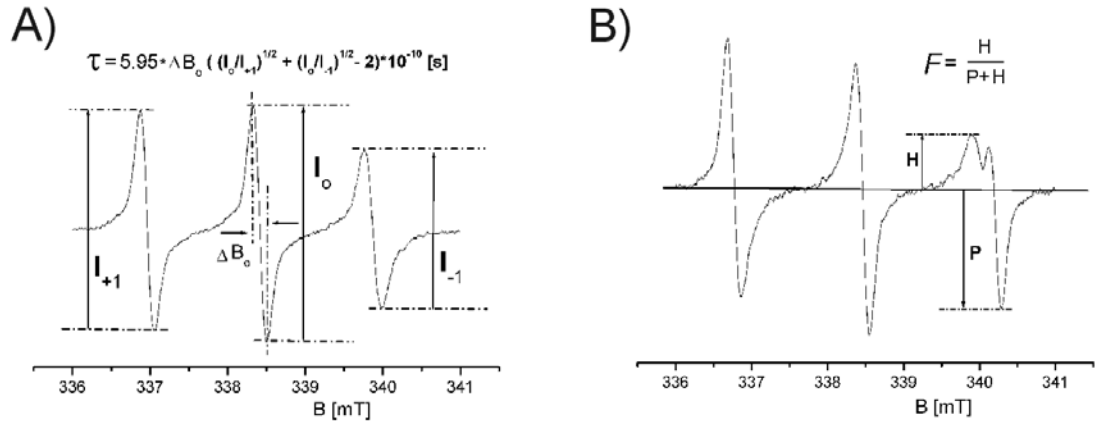

Fig. 2. ESR spectra of A) 16-DOXYL and B) TEMPO spin labels, (ΔB_0 in formula for τ is in gauss).

formed from egg yolk lecithin (EYL) upon addition of alanine and butyrine derivatives. The impact of small molecules on liposome membrane is well known, in particular on their fluidity and plasticity [6–8, 10, 11]. These studies related the impact of dopant concentration on the bilayer structure and mobility.

In the current studies we used Ac-Ala-NMe_2 and Ac-Abu-NMe_2 as dopants for the model EYL bilayer. The effect of such dopants on the plasticity and fluidity of bilayer was studied by ESR technique enhanced by typical spin probes – TEMPO and 16-DOXYL. The former probe penetrates both the hydrophobic interior and hydrophilic part of the bilayer while the latter one is selectively accumulated in the hydrophobic part of the bilayer only. Thus, the use of two spin probes enabled studies on bilayer mobility and fluidity both in the inner (hydrophobic) part and a polar water-lipid intermediate phase. The ratio of dopant to EYL varied from 0 to 25%.

Materials and methods

The egg yolk lecithin (EYL) was extracted from fresh eggs and purified according to the method described earlier [18]. Liposomes were obtained from EYL via the sonication process in distilled water using an ultrasonic disintegrator TECHPAN UD-20. To avoid overheating each 1 cm^3 of a sample was sonicated in six alternate cycles: 30 s of sonication and 30 s of cooling. The concentration of lecithin in the sample was 40 μM. A spin label with a concentration of 0.001 M relative to the lecithin (one spin probe particle per thousand molecules of lecithin) was added to the liposome water dispersion. Two spin probes, penetrating different regions of the membrane, were used in the ESR experiment – TEMPO and 16-DOXYL-stearic acid methyl ester.

The Ac-Ala-NMe_2 and Ac-Abu-NMe_2 dopants were obtained and described earlier [15, 19]. Their molecular structures are shown in Fig. 1. Small amounts of dopants were added to water suspension of liposomes, and all ESR measurements were conducted at room temperature (22°C). Spectra were recorded using the following instrumental settings – time constant – 0.3 s, modulation amplitude – 0.8×10^{-1} mT, scan time – 256 s.

The mobility of hydrophobic (inner) part of bilayer is studied by analyzing the ESR spectrum of 16-DOXYL probe (Fig. 2a) and the rotational correlation time τ. This parameter is related to degree of bilayer fluidity [17] and decreases with plasticity of the environment.

The ESR spectrum of TEMPO (Fig. 2b) allowed the determination of partition parameter *F*. This parameter describes membrane fluidity [5, 16] and is expressed as the ratio of amplitude of high field line of probe in water environment (*P*) to the low field signal originating from lipid surrounding (*H*). The increase of membrane fluidity is related to higher values of *F*. In contrary, the stiffening of membrane is observed upon decreasing of partition parameter *F*.

F and τ parameters of the studied systems provide detailed information on dynamic properties of the studied membrane as a function of dopant concentration. Each measurement was repeated three times and averaged results were used for the consecutive analysis. The relative errors in determined parameters *F* and τ were 2 and 3%, respectively.

Results and discussion

In Figures 3 and 4 are shown changes of relative spectroscopic parameters (F/F_0 and τ/τ_0) of TEMPO and 16-DOXYL spin probes in EYL bilayers on Ac-Ala-

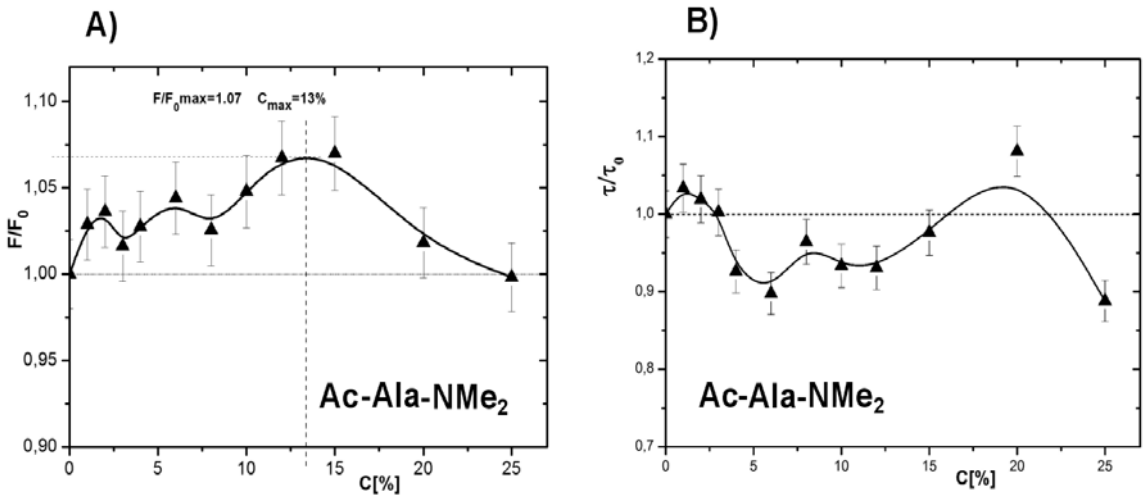

Fig. 3. The dependence of relative value of spectroscopic parameters on alanine dopant concentration (mol.%) (A) F/F_0 for TEMPO and (B) τ/τ_0 for 16-DOXYL.

-NMe_2 and Ac-Abu-NMe_2 dopant concentration. Pure bilayers are characterized by F_0 and τ_0 parameters.

In Fig. 3a are shown the relative changes of partition parameter F on alanine derivative concentration. An apparent, and nearly continuous, increase of fluidity of the membrane surface to the concentration of about 13% of Ac-Ala-NMe_2 is evident from Fig. 3a. Thus, the higher plasticity of EYL bilayer surface is observed for about 13% of alanine dopant and then the structure gets more rigid (to about 20% of Ac-Ala-NMe_2).

In Fig. 3b are shown the relative changes of rotational correlation time τ upon increasing alanine concentration. A relatively small impact of Ac-Ala-NMe_2 concentration on plasticity of bilayer was observed – the largest effect was for about 6% of dopant concentration. At a higher concentration of Ac-Ala-NMe_2 an increase of τ parameter was evident. This indicates the formation of more rigid interior of bilayer. The presence of extreme values in Fig. 3 indicates complexity of the studied processes due to "dissolution" and redistribution of alanine in EYL bilayer. The net impact of Ac-Ala-NMe_2 is observed by ESR as a complex change of spin probe mobility. The complex impact of organic dopants on the changes of spectroscopic parameters of ESR spin probes was reported earlier [8, 12–14]. One possible explanation of extremal changes of ESR parameters is due to the transport and introduction of electrical charge into the bilayer. Some theoretical attempts try to model such processes [9].

The increase of butyrine concentration up to 8% causes a subsequent loss of EYL bilayer rigidity at the membrane-water contact surface, demonstrated by an increase of the relative change of partition parameter F (Fig. 4a). Increasing the amount of butyrine (measured up to 25%) in the sample causes a continuous decrease of F parameter. A significantly higher impact of butyrine, as compared to alanine, is observed for inner, hydrophobic part of bilayer (Fig. 4b).

The increase of τ parameter in the studied range of butyrine derivative concentration (the rapid changes in the concentration ranges from 0 to 5% and without a clear maximum), indicates the decrease of membrane fluidity. An apparent local minimum for about 10% of Ac-Abu-NMe_2 is also observed. This is in agreement with a maximum of partition parameter F, observed in Fig. 4a. It is interesting to notice that butyrine derivative increases the fluidity of polar head surface groups (from changes of F parameter) and makes the interior

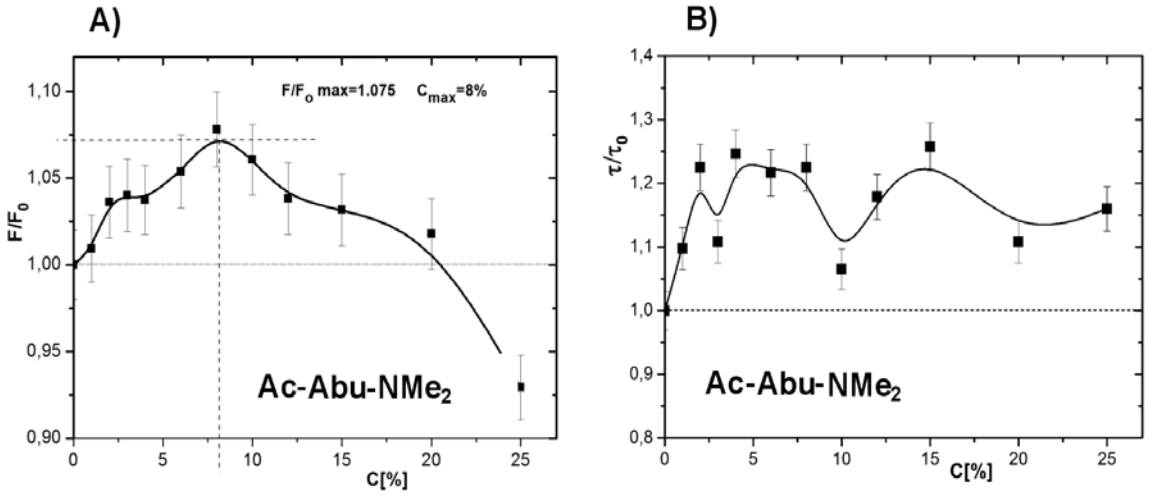

Fig. 4. The dependence of relative value of spectroscopic parameters on butyrine derivative dopant concentration (mol.%) (A) F/F_0 for TEMPO and (B) τ/τ_0 for 16-DOXYL.

of membrane more rigid (changes of τ parameter). In general, earlier studies reported similar changes in fluidity obtained for both the interior and surface of the bilayer. This strange discrepancy, if real, should be studied more thoroughly in the future works.

Conclusions

The current study leads to the following conclusions:

1. The introduction of both alanine and butyrine derivatives to EYL bilayers causes an increase of their surface fluidity in a non-monotonous manner (there was a clear maximum in changes of partition parameter *F*).
2. Ac-Ala-NMe_2 only slightly modifies the interior part of EYL bilayer and causes the increase of its fluidity (the $\tau/\tau_0 < 1$ in nearly entire studied concentration range). On the other hand, Ac-Abu-NMe_2 significantly increases the rigidity of EYL bilayer inner part ($\tau/\tau_0 > 1$ for all the studied concentrations).

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