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THE SELECTIVE INFLUENCE OF L- AND D-AMINO ACIDS ON THE BIOMEMBRANE

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The influence of L-Val and D-Val amino acids on a phospholipid bilayer, which serves as a physical model for the cell membrane, has been studied using the simultaneous small and large angle X-ray diffraction, as well as optical polarizing microscopy methods. It is shown that despite L-Val and D-Val amino acids having the same structure and differing only on their chirality, the effect of L-amino acids and, consequently, their permeability on the membrane is greater than that of D one. This indicates that the cell membrane exhibits a certain selectivity towards chiral molecules. Additionally, it was demonstrated that the hydrophobicity and hydration of an L- and D-amino acids also play a significant role on the interaction between the amino acids and the cell membrane.

It is known that L-amino acids are more prevalent in nature than D-amino acids. This research was to explore the interaction of L- and D-amino acids with the phospholipid bilayer, which can serve as a physical model of the biological membrane, aiming at understanding the differences of their effects. This could help to explain the widespread presence of L-amino acids in nature and the scarcity of D-amino acids.

The data obtained in this work suggest that this difference is likely due to the distinct mechanisms of interaction between L- and D-amino acids with the bilayer. Investigating the interaction of these amino acids with the phospholipid bilayer, which forms the essential structure of biological membranes, one can gain insights into the mechanisms of their interaction with biological membranes.

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***Keywords*:** L-Val and D-Val amino acids, selective effects on phospholipid bilayer, chiral molecules, hydrophobicity.

Introduction. In modern biology, membrane structures are widely prevalent, highlighting the crucial role of phospholipid bilayers in the emergence of life on Earth and suggesting the impact of the biophysical properties of membrane structures on cellular function. Similar structures and properties of molecules should facilitate molecule-membrane interactions and, consequently, permeability. The observation of the selective permeability might have been the most convincing evidences of a membrane recognition of a molecule. There are few studies in the literature [1–5] regarding the interactions of chiral molecules with biological membranes. The data

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on the interaction of amino acids with biological membranes are almost nonexistent. On the other hand, discovering the mechanisms of the interaction between chiral molecules and biological membranes might have allowed to fabricate lipid bilayers with desired properties.

Materials and Methods. To study phosphatidylcholine ("Avanti (polar lipids)", purity 93%)–water–amino acid systems on the molecular level, the X-ray diffraction method was employed. We used the L- and D-forms of L-Val ("Reanal", Budapest, purity 92.5%) and D-Val ("Reachim", Moscow, purity 91%) amino acid. Experimental data were obtained using the X-ray diffraction equipment DRON-3M and X-ray Diffractometer MD-10, equipped with specialized X-ray chambers of types PKCO and HOB, which allow simultaneous application of the X-ray diffraction method at both small and large angles.

When studying with the DRON-3M device, the diffracted beams at large angles are recorded on the X-ray film. The initial beam is absorbed by a lead plate located on the photographic box, where a film is positioned for recording reflections at small angles. This type of chamber allows recording of reflections in the range of 2Θ = 1.5–40°, with the small angle portion of 1.5–3°. The exposure time for X-ray imaging with the DRON-3M was 3–5 *h*.

In the case of the X-ray Diffractometer MD-10 device, the obtained diffraction pattern was recorded directly onto the computer, allowing necessary analysis. The exposure time was 15–20 *min*. For samples, capillaries made of quartz with their wall thicknesses of 0.1 *mm* and diameters of 0.4–1.0 *mm* (produced in Germany) were used, as well as "sandwich" type cells [6].

The phosphatidylcholine–water–amino acid ternary systems are prepared as follows: a weighed amount of dry phosphatidylcholine is gradually added, drop by drop, to double-distilled and deionized water and mixed vigorously until a homogeneous mass is obtained. Then, an amino acid portion with the appropriate concentration is added. To analyze the X-ray diffraction patterns, the Luzzati two-parameter equation was used:

$$d = l_0 + \left(k + \frac{2\mu l_0}{\rho S_0}\right)\frac{C_w}{C_l},$$

where d is the period for the identity of the liquid crystal structure; l_0 is the length of the molecule the lamella; k is the swilling coefficient; μ is the mass of a single molecule; S_0 is the surface per head; ρ is the water density; C_w and C_l are the water and lipid concentrations, respectively [7–9].

Studies on the over-molecular level were carried out using a Japanese MEIJI type optical polarizing microscope. In this case, the sample was prepared, dropping the prepared specimen onto a glass slide and covering it with a coverslip. The magnification of the microscope in this case was ×600. The optical image is recorded and analyzed by the computer [9].

Results and Discussion. To investigate the lamellar phase structure of the phosphatidylcholine–water system, we used the L- and D-forms of L-Val and D-Val amino acids at neutral pH [10, 11]. Fig. 1 shows the effects of these amino acids on the curve, representing the dependence $\sim \frac{C_w}{C_l}$.

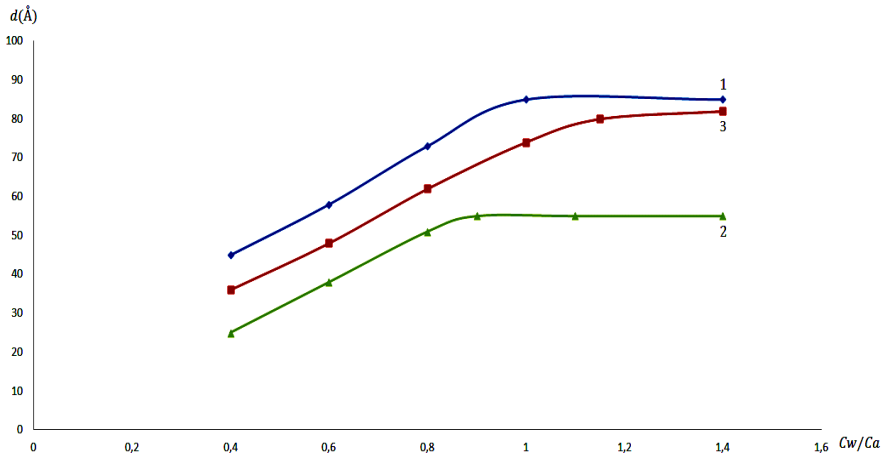
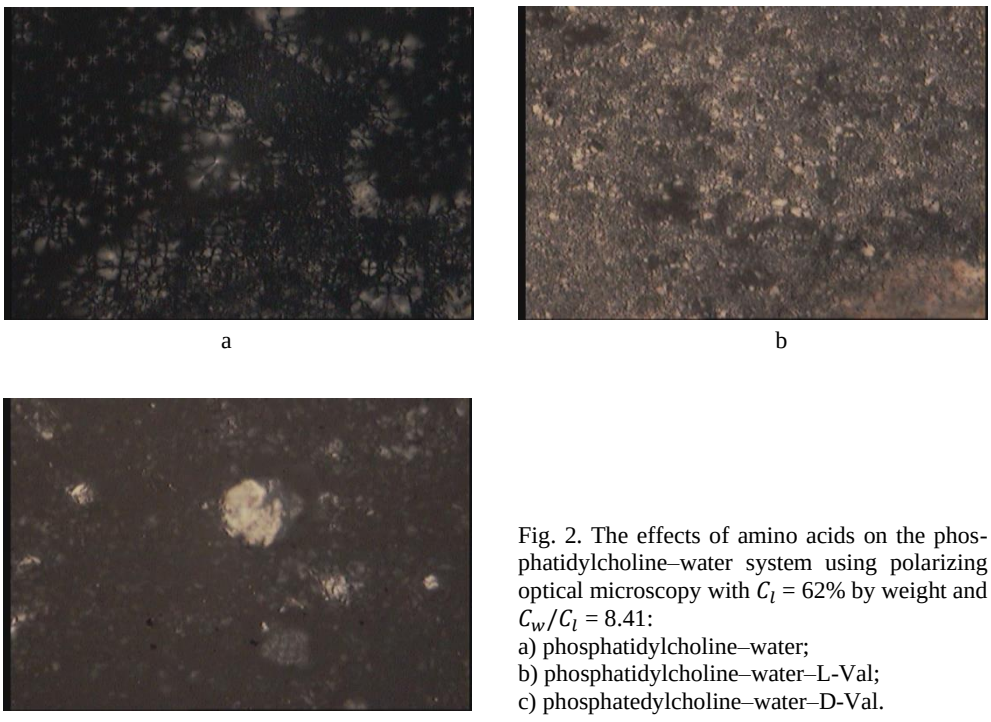


Fig. 1. Dependence $d \sim C_w/C_l$ for the lamellar phase of the phosphatidylcholine–water system in the presence of amino acids: 1) phosphatidylcholine–water; 2) phosphatidylcholine–water–L-Val; 3) phosphatidylcholine–water–D-Val, where C_w/C_l =8,41.

To study the effects of amino acids on phosphatidylcholine–water systems, using polarizing optical microscopy, the amino acids L-Val and D-Val were used in this case.



a b c

Fig. 2. The effects of amino acids on the phosphatidylcholine–water system using polarizing optical microscopy with C_l = 62% by weight and C_w/C_l = 8.41:
a) phosphatidylcholine–water;
b) phosphatidylcholine–water–L-Val;
c) phosphatedylcholine–water–D-Val.

Fig. 2, b shows the micellar structures formed at 58% concentration. When L-Val was introduced to the phosphatidylcholine–water system, the micellar

structures were preserved, but their quantity increased, and their appearance became somewhat blurred. The increase in the number of micelles is due to a change in the critical concentration for micelle formation caused by the effect of L-Val. The loss of structural clarity in the micelles is ascribed to changes in the balance of electrostatic and van der Waals interactions, which cause them to self-organize. In the case of D-Val on the phosphatidylcholine-water system, there was the minimum effect. As seen in Fig. 2, c, D-Val does not interact with this system; instead, it forms a separate phase. This may be due to the relatively low hydrophobicity of the amino acid and the specific orientation of its dipole fragment within the phospholipid bilayer (due to the direction and magnitude of the angle formed with the bilayer).

This conclusion can be drawn based on the analysis of the results obtained from X-ray diffraction measurements.

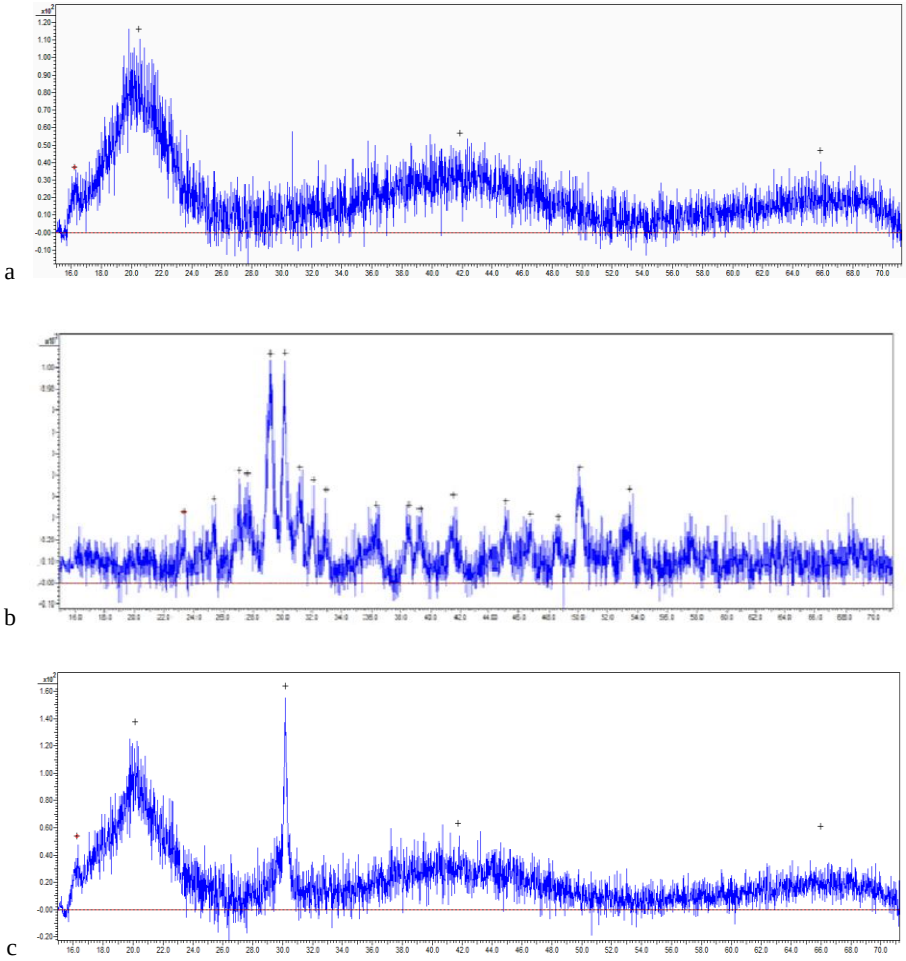


Fig. 3. X-ray diffraction patterns of the lamellar phase of the phosphatidylcholine–water system in the presence of amino acids with C_l = 62% by weight and C_w/C_l = 8.41: a) phosphatidylcholine–water; b) phosphatidylcholine–water–L-Val; c) phosphatidylcholine–water–D-Val.

As seen in Fig. 3, the L-Val results in multiple reflections at different angles, which are due to the presence of micelles with varying degrees of hydration. Comparison of the diffraction patterns on Figs. 3, a and 3, c shows that two peaks of the phosphatidylcholine–water system are reproduced, but with lower intensity and increased half-width. This indicates a weak effect of D-Val on this system. The sharply defined peak with a small half-width between these two peaks suggests the presence of a separate crystalline phase of D-Val. From these results, it can be concluded that there is a selective interaction with the lamellar phase. Anyhow, amino acids lead to a decrease in the slope of the linear region of $d \sim C_w/C_l$ curve, as well as an increase in the C_w/C_l ratio (Fig. 1), at which point d reaches a constant value. Thus, amino acids also contribute to the increase in the swelling coefficient of phosphatidylcholine in water. Additionally, the effect of amino acids causes internal disruption within the lamellae.

Such effect of amino acids on the structure of the lamellae can be achieved, if there is dipole-dipole interaction between the dipole fragments of the amino acids and phosphatidylcholine molecules. This interaction increases the dipole moments of the phosphatidylcholine molecules and alters their polarizability. With an increase in molecular hydrophobicity, the collinearity of the dipoles of amino acids and phosphatidylcholine can disrupt in favor of the contact between the hydrophobic part of the amino acid and the hydrocarbon domain of the lamella. This leads to a reduction in the overall dipole moment of the molecule, as well as a decrease in the hydrophilicity of phosphatidylcholine, which, in turn, causes disruption of the lamellae.

Conclusion. Based on the obtained data, the following conclusions can be made.

1. The phospholipid bilayer exhibits selective interaction with the L- and D-forms of amino acids.

2. This may provide a basis for understanding, why the L- and D-forms exhibit different permeability properties through the membrane.

3. The difference in the interactions of the L- and D-forms with the lipid–water system can be explained by the orientation of the dipole fragments in the lipid bilayer and the reduction of the system's hydrophobicity.

4. Discovering the mechanisms of interaction between chiral molecules and biological membranes can provide an opportunity of creating lipid bilayers with desired properties.

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ԿԵՆՍԱԹԱՂԱՆԹԻ ՎՐԱ L ԵՎ D ԱՄԻՆԱԹԹՈՒՆԵՐԻ ԸՆՏՐՈՂԱԿԱՆ ԱԶԴԵՑՈՒԹՅՈՒՆԸ

Ռենտգենյան ճառագայթների միաժամանակյա փոքր և մեծ անկյունների տակ դիֆրակցիայի և օպտիկական բևեռային մանրադիտակի մեթոդներով ուսումնասիրվել է L և D ամինաթթուների ազդեցությունը կենսաթաղանթի ֆիզիկական մոդել հանդիսացող փոսֆոլիպիդային երկշերտի վրա: Ցույց է տրված, որ, չնայած այն բանին, թե L և D ամինաթթուներն ունեն նույն կառուցվածքը և տարբերվում են միայն քիրյալությամբ, L ամինաթթուների ազդեցությունը և դրա հետևանքով թափանցունակությունը ավելի մեծ է, քան D ամինաթթվի դեպքում: Այսինքն՝ կենսաթաղանթը ցուցաբերում է որոշակի ընտրողականություն քիրյալ մոլեկուլների հանդեպ: Ցույց է տրված, որ L և D ամինաթթուների և կենսաթաղանթի փոխազդեցության մեջ կարևոր ներդրում ունեն նաև դրանց հիդրոֆոբությունը և հիդրատացիան:

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ИЗБИРАТЕЛЬНОЕ ВОЗДЕЙСТВИЕ L- И D-АМИНОКИСЛОТ НА БИОМЕМБРАНУ

Методом рентгеновской дифракции одновременно под малыми и большими углами, а также методом оптической поляризационной микроскопии исследовано взаимодействие L- и D-аминокислот с фосфолипидным бислоем, являющимся физической моделью биомембраны. Показано, что, несмотря на то что L- и D-аминокислоты имеют одинаковую структуру и различаются только хиральностью, воздействие и проницаемость L-аминокислот больше, чем D-аминокислот. То есть биомембрана проявляет определенную селективность по отношению к хиральным молекулам. А также показано, что гидрофобность и гидратация L- и D-аминокислот играют важную роль во взаимодействии между ними и биомембраной.