Abstract

Various substitutes of the biological membranes are used in the structural studies of membrane proteins. The most commonly used are micelles, bicelles, and liposomes, but recently also nanodiscs become a frequent choice.

Nanodiscs are models made of a lipid bilayer, stabilized by the helical protein MSP1 (Membrane Scaffold Proteins), a modification of the naturally occurring ApoA I protein. The length of MSP1 proteins can be modified by multiplying internal helices. It allows obtaining nanodiscs in the diameter range from 6 nm up to 17 nm. An essential feature of nanodiscs is the ability to determine the number of lipids in the model precisely. They are stable and repeatable models with uniform particle size. These unique properties of nanodiscs make them applicable in many studies of membrane proteins. Appropriate selection of the model diameter enables investigating a single membrane protein and more complex systems as protein dimers, trimers, and more.

The properties of the lipid membrane, e.g., its lipid composition, significantly depend on its structure. It also plays a crucial role in the bilayer in nanodiscs. The presence of cholesterol in the bilayer is significant, as it is an essential ingredient for eukaryotic cells and has many different functions. Therefore, its occurrence in models used in structural research is often crucial. Unfortunately, its hydrophobic nature and difficulties with solubility in an aqueous environment significantly limit its use in biochemical investigations. An alternative to cholesterol is structural substitutes that have a higher solubility in the polar solutes.

One of the most commonly used cholesterol substitutes is cholesterol succinate (CHS) and DC-cholesterol (DC-CHL). The CHS is an ester of cholesterol and succinic acid. This change makes the molecule more soluble in water, facilitating its use in numerous studies, such as the crystallization of membrane proteins. In an environment with a pH of 7, it has an anionic form. The second substitute, DC-CHL, differs from cholesterol by attaching the acid derivative of N, N-dimethylaminoethanol to the hydroxyl group. It also promotes better solubility. At neutral pH, DC-CH has a cationic form. It causes frequent use of this steroid in liposomes as drug carriers or transfection of nucleic acids.

Scientific literature shows that the results obtained with CHS can be regarded as results describing cholesterol's effects. However, there is no such research for DC-CHL. Each structural modification of cholesterol changes its impact on the lipid bilayer, which causes a decrease in the order parameter and condensation of the membrane. Unfortunately, there are not so many studies that directly compare cholesterol and its substitutes.

This research aims to determine cholesterol substitutes' influence on the lipid bilayer structure and dynamics compared to cholesterol-containing models. Moreover, nanodiscs' characteristics compared to the biological membrane's ("flat bilayer") have been done. To achieve these goals, molecular modeling methods were used to study these structures at the atomic level. The characteristics of the studied models include analyzes of:

- The structure of the MSP1 protein,
- Physicochemical parameters describing a lipid bilayer,
- Description of the differences between nanodiscs and flat bilayer,
- Description of the differences between the influence of cholesterol and its analogs on the lipid bilayer.

The results show significant differences between nanodiscs and flat bilayer. The lipid bilayer in nanodiscs is heterogeneous due to the influence of the MSP1 protein. This protein significantly influences the lipid behavior in the model, which causes an overall lower order of the lipids in the lipid bilayer than in the flat bilayer. In the proximity of the MSP1 protein, lipids are less ordered than in the center of the nanodiscs, and the Scd order parameter present gradient value towards the center of the disc. The lipid bilayer has the smallest thickness next to the MSP1 protein. These results emphasize the existence of a different class of lipids in nanodiscs, which, depending on the distance from the nanodisc center, have different properties. The nanodisc's size is also essential - the larger nanodisc, the lower the lipid's overall order in the disc. Moreover, in bigger nanodiscs, the lipids population located in the center is less influenced by the MSP1 protein. These results describe the differences from the flat bilayer model, which is characterized by homogeneity in the lipids' properties in the model.

Obtained results more precisely characterize the effect of cholesterol on the lipid bilayer of nanodiscs, which cannot be studied using traditional experimental methods. The impact of cholesterol in nanodiscs is substantial and comparable to that observed in liposomes or the flat bilayer. Cholesterol organizes lipids in the lipid bilayer of nanodiscs. In nanodiscs containing cholesterol, the POPG lipids show a preference for locating in the close area of the MSP1 protein. Moreover, results show that the models' heterogeneity is caused by the influence of the MSP1 protein on the lipid bilayer. These observations are the starting point for research on cholesterol substitutes such as CHS and DC-CHL, mostly used experimentally as an alternative to this sterol. No studies have been performed to show the effect of these steroids on the lipid bilayer and compare them with the properties of models with cholesterol.

The cholesterol substitutes investigated in this study do not fully reflect the cholesterol's effect on the lipid bilayer. Firstly, CHS prefer localization in the close area of MSP1. It is the

consequence of the electrostatic interactions between this steroid with positively charged MSP1 residues. These interactions affect all lipids in nanodiscs what is in the opposite observation compared to the properties of nanodiscs with cholesterol. Secondly, in these discs, the POPG lipids also showed a preference for localization in the close area of the MSP1. The lipid bilayer of these models is also heterogeneous due to the effect of MSP1 protein on the disc. The results show that CHS is not recommended as a cholesterol substitute.

Another cholesterol substitute, DC-CHL, was also investigated. The results show that the steroid changes the properties of the lipid bilayer. It increases the overall order of lipids in investigated models. Moreover, even for these discs, the MSP1 has an impact on the heterogeneity of the lipid bilayer. Additionally, DC-CHL influences the distribution of the POPG lipid in the models. DC-CHL and POPG form aggregates in the central part of the nanodiscs. The number of interactions between these lipids is higher than the number of interactions observed in models with other steroids. It causes additional inhomogeneity of the lipid bilayer. Moreover, this steroid should not be used in models containing negatively charged lipids such as POPG. Nevertheless, DC-CHL may well reflect cholesterol behavior.

To summarize, the results obtained in this study provide a more detailed characterization of nanodiscs, particularly the influence of cholesterol substitutes on the properties of the lipid bilayer in various models and the description of its dynamics. Nanodiscs are exciting models that are more and more popular in structural research. However, the results show these models' essential properties, which should be considered in experiments design. First, the addition of the negatively charged lipids, such as POPG, influences the lipid bilayer's properties in nanodiscs and is not recommended in these models. Secondly, the selection of an appropriate cholesterol substitute is also crucial. CHS is not a suitable replacement because it does not reflect the cholesterol's effects on the lipid bilayer. DC-CHL is a better option; however, it should not be used in models containing POPG or other negatively charged lipids. The results clarify how the nanodiscs and cholesterol substitutes' properties can affect the membrane proteins investigated in these models. It provides more information, which will be very useful for structural studies. Molecular modeling methods used in this study allowed the investigation of mechanisms at the atomic level and the femtosecond time resolution. Obtained MD results complement and detail the results of research on nanodiscs derived from experimental methods.