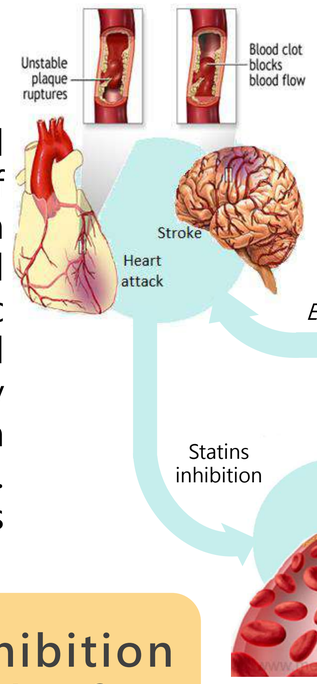
Inhibition effect of statins on HMG-CoA reductase reconstituted in model lipid rafts

Michalina Zaborowska[1], Dorota Matyszewska[2], Renata Bilewicz[1]
[1]Faculty of Chemistry, University of Warsaw, Warsaw, Poland
[2]Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland

INTRODUCTION

Hypercholesterolemia is a condition of elevated cholesterol blood levels that is often dangerous to human health. It promotes diseases of the cardiovascular system, causing heart attack or stroke. The key protein in the process of cholesterol synthesis is 3-hydroxy-3-methyl-glutaryl coenzyme A (HMGR). It is located in the membranes of the endoplasmic reticulum which are rich in cholesterol and sphingolipids forming lipid rafts. Statins are widely used to prevent cardiovascular diseases by inhibiting the catalytic site of HMG-CoA reductase. The HMGR protein has been reconstituted in the model lipid membrane (proteoliposomes). For this purpose, the DOPC:Chol:SM system, typical for lipid rafts, was selected.

The aim of this study is to investigate the inhibition effect of statins on HMGR presence in model lipid rafts



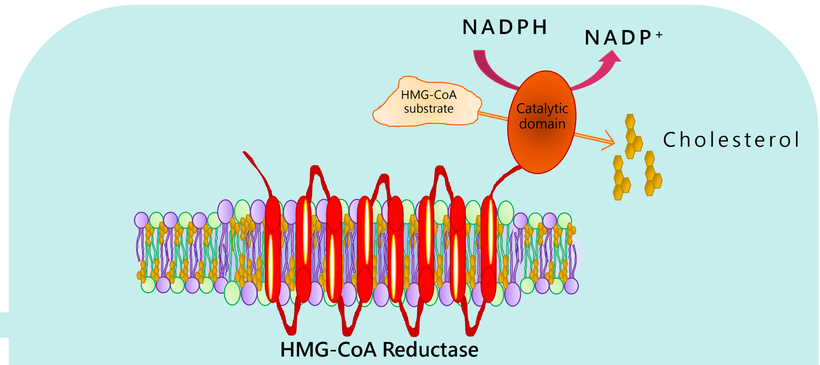


Fig. 1. Schematic representation of the reactions involving the studied enzyme – HMG-CoA reductase

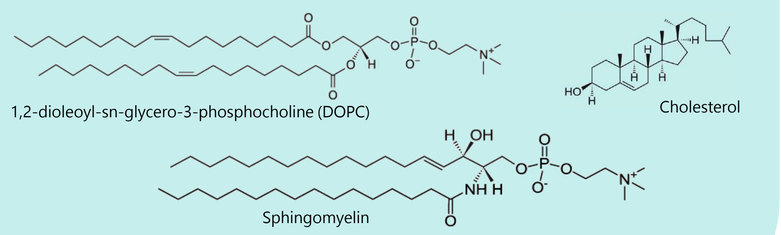


Fig. 2. Chemical structures of lipids characteristic for lipid raft of endoplasmic reticulum

Characteristics of liposomes and proteoliposomes

Dynamic light scattering (DLS) and fluorescence microscopy

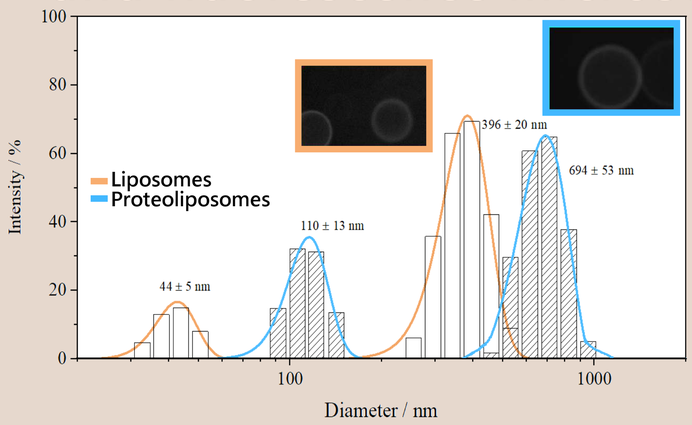


Fig. 3. Calculated Gaussian distributions of the particle sizes as determined with dynamic light scattering (DLS) for DOPC:Chol:SM 1:1:1 liposomes and proteoliposomes with HMG-CoA reductase and visualization of vesicles doped with NBD-cholesterol by fluorescence microscopy.

- The Bangham method allows to obtain vesicles composed of DOPC:Chol:SM.
- Direct reconstitution of the reductase HMG-CoA results in the double expansion of the structure.

CONCLUSIONS

1. HMG-CoA reductase was effectively reconstituted into a lipid vesicles, which resulted in well-defined proteoliposomes.
2. The study of surface properties and electrochemical tests have shown that reductase by incorporating into the DOPC:Chol:SM layer causes its expansion.
3. The protein incorporation process into liposomes did not result in the loss of activity of the protein catalytic site.
4. Statins inhibit the enzyme's catalytic site, and thus stop the cholesterol-producing reaction in the human body.

Effect of statins inhibition

UV-Vis activity measurements

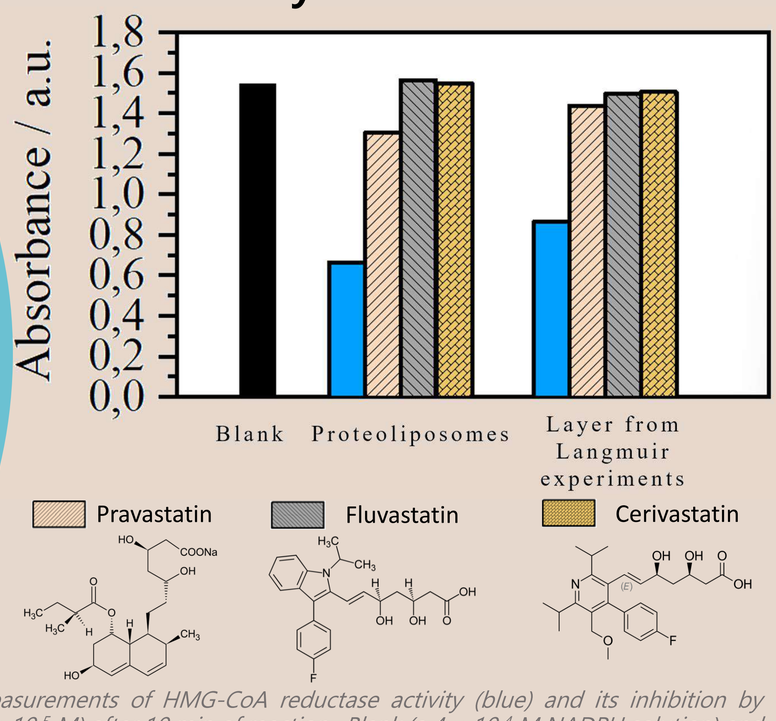


Fig. 5. Measurements of HMG-CoA reductase activity (blue) and its inhibition by statins (1×10^{-5} M) after 10 min of reaction. Blank (a 4×10^{-4} M NADPH solution) and chemical structures of statins

- In the absence of HMG-CoA reductase catalyzing the reaction, the maximum absorbance value is 1.6
- Decrease in the absorbance value in the presence of the reductase in the proteoliposomes and the lipid layer indicates that HMG-CoA reductase works properly and fulfills its enzyme role in the system
- Statins inhibit HMG-CoA reductase (absorbance value increases), thus stopping the reaction of cholesterol production in hepatocytes

Langmuir Experiments

- The change in the isotherm at high surface pressures indicates a reorganization of the structure or a partial collapse
- The isotherm obtained for the proteoliposomes shows a strong expansion of the layer after protein incorporation and thus its fluidization
- Given amount of protein (0.02% mole) in the proteoliposomes has a stabilizing effect on the layer (~ 22 mN/m, blue curve, right)

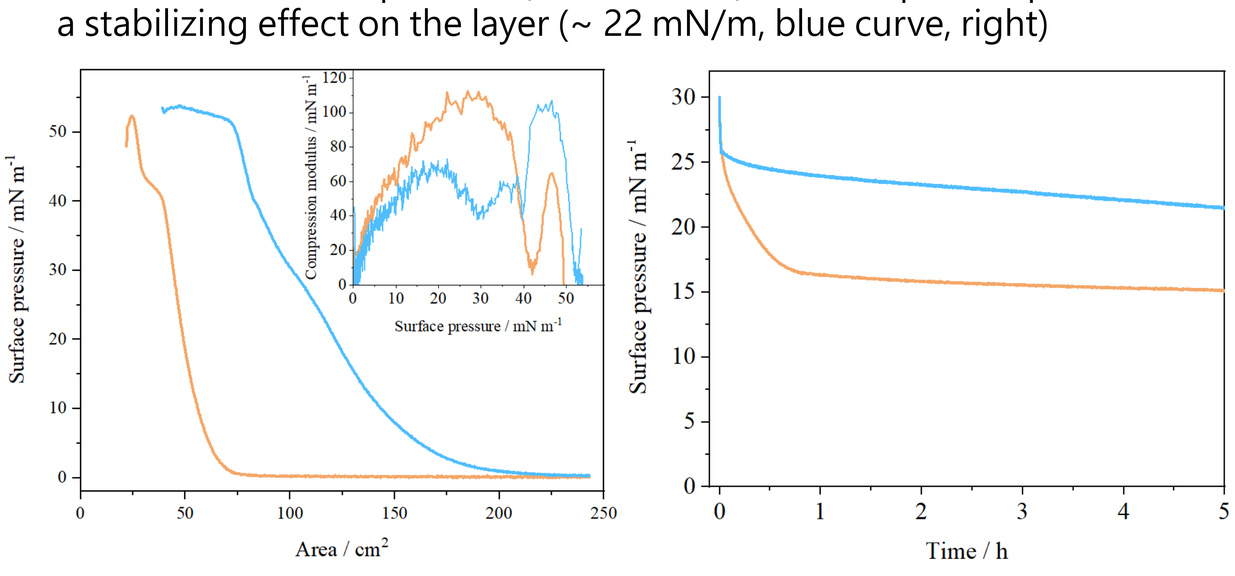


Fig. 4. Surface pressure – area per molecule (π-A) isotherms of DOPC:Chol:SM 1:1:1 layers of liposomes (orange) and proteoliposomes with HMG-CoA reductase (0.02 mol%) (blue) and the surface pressure vs. time plots. Left inset: compression modulus vs surface pressure plot. (T=21±1°C)

Linear Sweep Voltammetry (LSV)

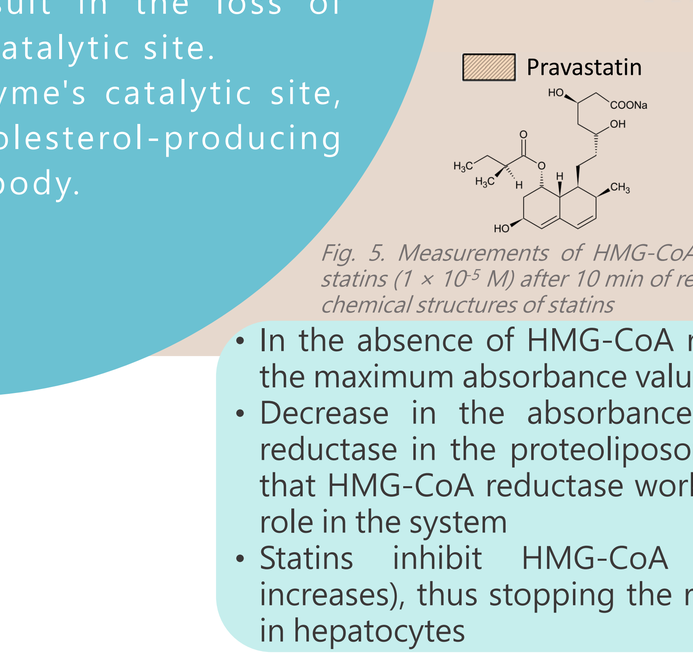


Fig. 6. The dependence of the current of the NADPH oxidation peak indicating the activity of reductase and its inhibition by statins on the duration of the reaction

- The catalytic site of reconstituted reductase shows activity
- The degree of inhibition depends on the degree of penetration of the proteoliposomes by statins
- Statins inhibit the HMGR catalytic site so the NADPH concentration in the solution remains constant - the peak current of its oxidation on the electrode does not decrease with time

WHAT'S NEXT?

The aim of the research has been achieved, but the question still remains - how is HMG-CoA reductase located in the model biological system?

References

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