The β-cyclodextrins as carrier for target delivery of pharmaceutical substances against lipase from *Malassezia spp*.

V. A. Filatov, Lomonosov Moscow State University, Russian Federation

E-mail: filatovviktor097@gmail.com

INTRODUCTION AND AIM OF STUDY

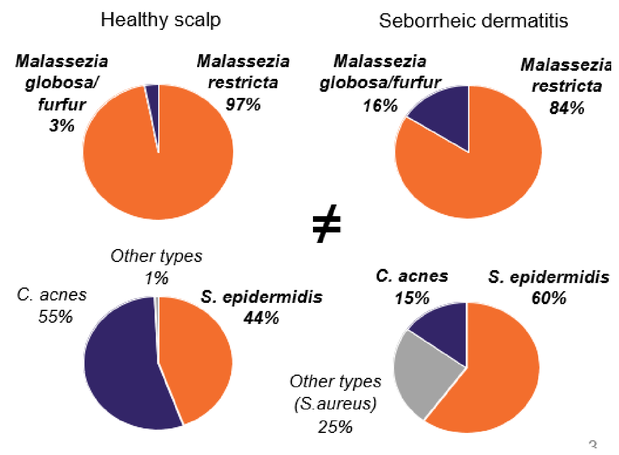
According to statistical data, seborrheic dermatitis (SD) and dandruff are the most common dermatological diseases in more than 50% of the population all over the world. *Malassezia species* have a main role in SD pathogenesis. Interestingly, *Malassezia species* secrete several lipases that are responsible of the production of free fatty acids and inflammatory cytokines. The **lipase** is considered as new potential target for SD therapy because it is essential enzyme for fungi vital activity

A completely new approach based on application natural β-cyclodextrines as a carrier for target delivery of pharmaceutical substances against lipase was suggested. The **β-cyclodextrins** (β-CD) are cyclic oligosaccharides that enhance stability of phytochemicals and provide delivery of substances. CDs can form various inclusion complexes with lipase and act as carrier of lipase inhibitors. In present study, the effects of β-CD on lipase structure and enzymatic hydrolysis of acylglycerides by lipase were investigated by modern methods.

The prevalence of seborrheic dermatitis in different countries of the world

Scalp Care Exploration Study 2020, 5 countries
CICS database 2016-2018, 20-22 countries

Scalp microflora changes during seborrheic dermatitis



The present study aimed to evaluate influence of β-CD on lipase structure and enzymatic activity for hydrolysis of acylglycerides by lipase from *Malassezia spp.*

MATERIALS AND METHODS

Lipase isolated from *Malassezia restricta* and purified β-cyclodextrins (Zhongbao Chemicals Co., Limited)

1. Lipase Assay Activity

It was performed according to the method described in articles. 5 g of glyceryl oleate (substrate) was emulsified in 95 mL of distilled water with 2% polyvinyl alcohol, homogenized for 6 minutes. 1 ml of solution containing lipase (40-80 g/L) and β-CD (0-49.94 mg/mL) was added to 5 mL of substrate and diluted to 10 mL with phosphate buffer (pH 7,0-7,4). Conditions: 25±2*C for 15-180 minutes. Ethanol (15 mL) was added to finish the reaction. After all, mixtures was titrated with NaOH (50 mM) using phenolphthalein. The rate of hydrolysis (%) was calculated using formulas:

C – the concertation of NaOH (0.05 mol/L)
M – the sample weight (g)
V – the NaOH solution volume for titration (mL)
AV – the acid value (mg NaOH/g)
SV – the saponification value (mg NaOH/g)

$$AV = 39.99 * C * M/V \qquad X\% = \frac{AV_1 - AV_0}{SV - AV} \times 100\%$$

2. Ultraviolet (UV) Spectroscopy Analysis

The UV spectrum of the homogenous dispersion of lipase (1.67 mg/ml) and β-CD (0-4.54 mg/ml) was measured by a Specord 250 Plus ultraviolet spectrophotometer (190-340 nm).

3. Fluorescence Spectroscopy Analysis

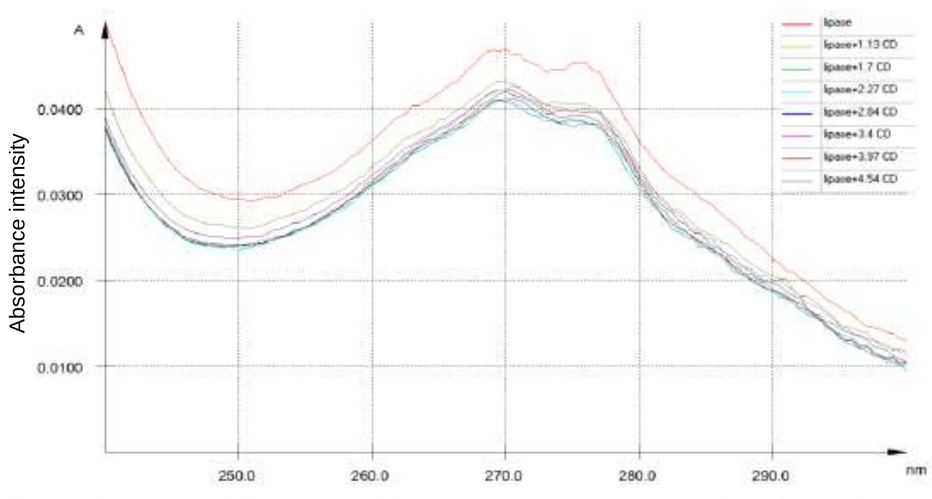
The fluorescence spectrum was measured with Fluorolog-3 fluorescence spectrophotometer at 280 nm (excitation) and 300-500 nm (emission). Lipase (1.67 mg/ml) and β-CD (0-4.54 mg/ml) were dissolved into Tris-HCl buffer (0.05 mol/L, pH 7.7).

4. Transmission Electron Microscopy Analysis

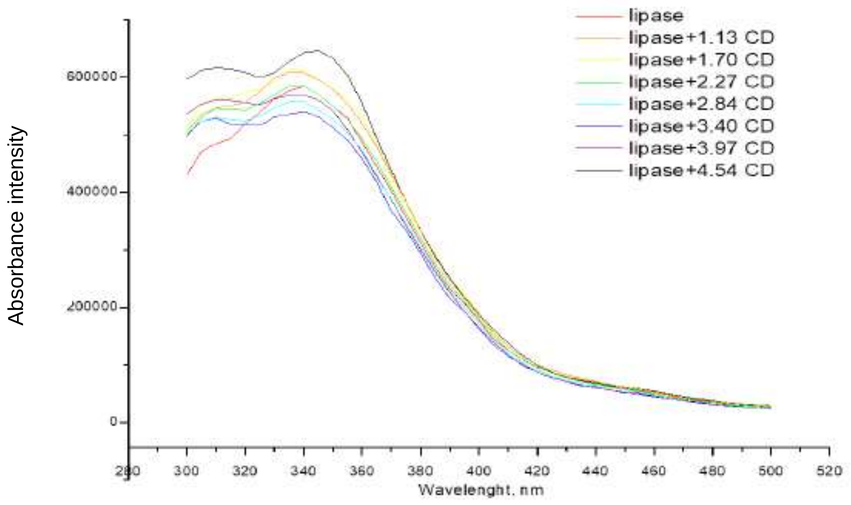
Morphological analysis of the dried complex lipase with β-CD was carried out using FEI Tecnai G2 F20 S-TWIN TMP equipped with a STEM detector. Accelerating voltage 80, 200 kV, extraction voltage 4500 V.

RESULTS AND DISCUSSION

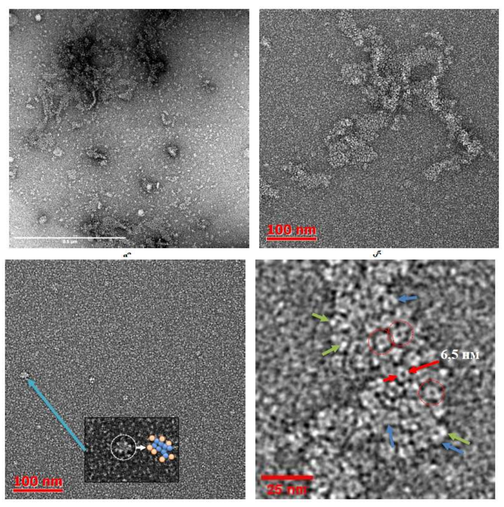
UV absorption revealed about surface interaction and formation of clathrate-like inclusion complex of lipase with β-CD (4.54 mg/mL) due to interaction between aromatic amino acids as tryptophan and tyrosine from lipase surface with β-CD.



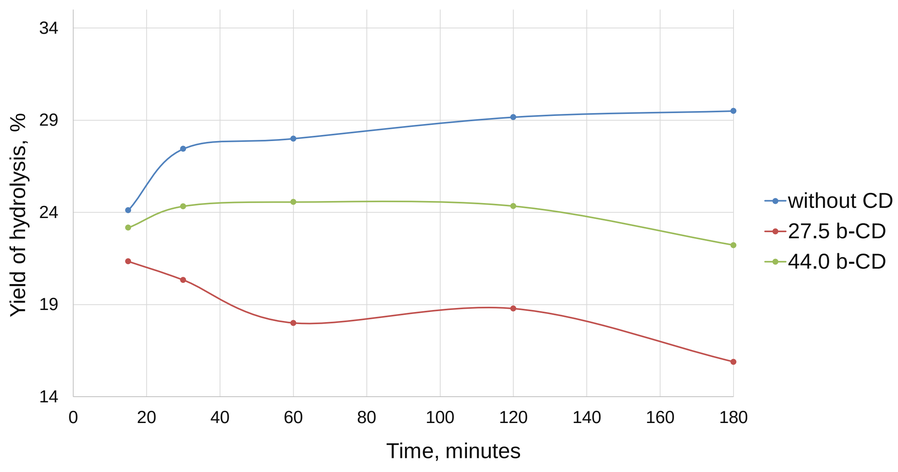
Fluorescence analysis showed increase the emission intensity of lipase in presence of β-CD (0-1.7 mg/mL and 3.4-4.54 mg/mL) due to interaction between tryptophane in active center of lipase with β-CD.



Lipase formed grape-like active conglomerate with β-CD molecules that changed enzymatic activity.



β-CD decreased lipase activity up to 35% compared to β-CD-free lipase samples after 1 hour. Lipase (40 mg/mL) produced the lowest yield of oleic acid in presence of β-CD (27.5 mg/mL) than in presence of β-CD (44.0 mg/mL).



CONCLUSIONS

1. UV spectroscopy and fluorescence analysis showed that β-CD interacted with surface and active center of lipase in different concentrations.
2. Lipase formed a special grape-like complex with β-CD that can be helpful for target delivery of lipase inhibitors.
3. The enzymatic activity of the lipase against acylglycerides decreased in presence of β-CD molecules that can be useful for inhibition of enzyme activity.
4. β-CD can be used as carrier of natural and synthetic lipase inhibitors for successful treatment of seborrheic dermatitis and good stability of formulations with these substances.

ACKNOWLEDGMENT

This research work was funded and carried out jointly with the Science Center of Splat Global LLC.