Experimental report

14/02/2018

Proposal: 8-02-787 **Council:** 4/2016

Title: Membrane lipid composition and antifungal drug resistance in pathogenic yeast

Research area: Biology

This proposal is a new proposal

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Samples: Silicon blocks
deuterated yeast lipid extract from Candida glabrata
yeast lipid extract from Candida glabrata

Instrument	Requested days	Allocated days	From	To
D17	7	0		
FIGARO	7	4	15/12/2016	19/12/2016

Abstract:

The yeast Candida glabrata has became a main cause of mucosal and systemic infections during recent decade, but its virulence and resistance factors are so far only poorly understood.We have recently developed a set of RNA interference tools that allow us to study the virulence and resistance mechanisms in C. glabrata at the genetic level by upregulating/downregulating specific genes, as well as the lipidomic tools to determine the corresponding changes in membrane lipid and sterol composition. This project builds on the results obtained in our recent study using membranes from the model yeast Pichia pastoris, which showed that the AmB mechanism of interaction is strongly influenced by both the phospholipid and sterol composition of the membranes. The aim of this proposal is to investigate the role of the different lipid components of C. glabrata membranes and the structural basis of AmB activity in membranes derived from two C. glabrata strains manipulated with our RNAi tools so that they are more susceptible or resistant to AmB respectively than the wild type.

Experimental Report
FIGARO - 8-02-787

Abstract:

The yeast *Candida glabrata* has become an increasingly important cause of mucosal and systemic fungal infections during the recent decade, but its virulence and resistance factors are so far much less well understood in comparison to the more common *Candida albicans*. We have recently developed lipidomic methods to determine changes in membrane lipid and sterol composition in C. glabrata in response to changes in the regulation of virulence and resistance genes, using a set of RNA interference tools that allow us to upregulate/downregulate specific genes. This project builds on the results obtained in our recent study using membranes from the model yeast *Pichia pastoris*, which showed that the mechanism of the antifungal agent amphotericin B (AmB) is strongly influenced by both the phospholipid and sterol composition of the yeast membranes. The aim of this experiment was to investigate the structural basis of AmB activity in membranes derived from two *C. glabrata* strains manipulated with our RNAi tools so that they are either more susceptible or resistant to AmB respectively than the wild type clinical isolate.

Results:

During the 4 days allocated out of the 7 days we requested in the proposal, we measured the solvent contrasts and samples listed below (Table 1). We fully characterized the total lipid extracts of three *C. glabrata* strains with a different sensitivity towards AmB. The strain Y2296 (transformed with an empty vector) was used as reference, whereas Y2311 was found to be more sensitive and Y2310 more resistant toward AmB, as judged by the MIC (minimum inhibitory concentration) values.

Bilayers	Contrasts before AmB addition	Contrasts after AmB addition
hTotal extract from Y2296 (empty vector)	D_2O, Cm4, CmSi and H_2O	D_2O, Cm4 and H_2O
hTotal extract from Y2310 (AmB resistant)	D_2O, Cm4, CmSi and H_2O	D_2O, Cm4 and H_2O
hTotal extract from Y2311 (AmB sensitive)	D_2O, Cm4, CmSi and H_2O	D_2O, Cm4 and H_2O
dTotal extract from Y2296 (empty vector)	D_2O, Cm4, CmSi and H_2O	D_2O, Cm4 and H_2O
dTotal extract from Y2310 (AmB resistant)	D_2O, Cm4, CmSi and H_2O	D_2O, Cm4 and H_2O
dTotal extract from Y2311 (AmB sensitive)	D_2O, Cm4, CmSi and H_2O	D_2O, Cm4 and H_2O

Table 1. List of samples and solvent contrasts measured. hTotal refers to the total lipid extract from each strain grown in H_2O, whereas dTotal refers to the total lipid extract oft each strain grown in 100% D_2O using d_8-glycerol as the carbon source. Cm4 = 66%D_2O, CmSi = 38% D_2O, mixed volumetrically using an HPLC pump.

The neutron reflectivity curves measured on FIGARO, using 2-30 Å as wavelength band and 7% chopper resolution, before and after AmB addition on the total lipid extract bilayers, showed different responses for the different strains. The data from the deuterated samples, in which the differences can be most clearly observed, are presented in Figure 1. In the case of the AmB sensitive Y2311 membrane, the reflectivity at low q ($q \leq 0.05$ Å^{-1}) increased particularly in the most sensitive contrasts H_2O and CM4 in comparison to the contrasts measured before AmB addition, while for the AmB resistant Y2310 membrane, the reflectivity decreased below the ones measured before adding AmB. These relative changes in contrast are consistent with observations made in the corresponding hydrogenous bilayers. In the case of the deuterated AmB-sensitive strain Y2311, the best fits to the data indicate that AmB inserts into the lipid chain region at a volume fraction of 11 ± 2 % v/v and very similarly in the hydrogenous bilayer from Y2311. A similar AmB layer formed on top of both

membranes in terms of its thickness (18 ± 3 Å) and hydration (90 ± 3 % v/v). In the bilayer from the AmB resistant strain Y2310, the amount of AmB inserted in the in the hydrophobic chains (2 ± 1 % v/v) was considerably smaller. The changes that occur in the lipid chain scattering length densities could be interpreted in a way that corresponds to almost all the ergosterol removed in the case of the sensitive Y2311 strain, but only a marginal extraction in the resistant Y2310 strain. However, in both fully hydrogenous or fully deuterated samples the contrast between the lipids and ergosterol is relatively low and the results need to be confirmed by recording data using sample in which deuterated ergosterol is reconstituted into the hydrogenous lipid extract and vice versa, in a manner similar to our previous data on *P. Pastoris* membranes (experiment report no. 8-02-773).

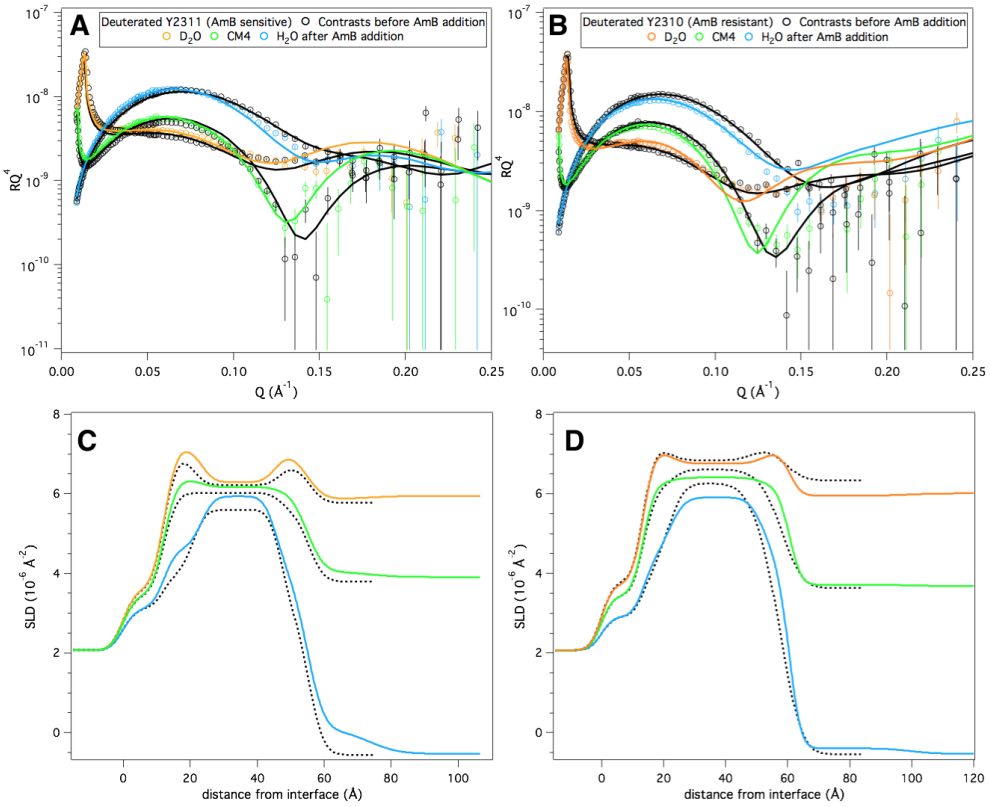

Figure 1. Comparison of the neutron reflectivity data, fits (A & B) and associated scattering length density (SLD) profiles of the total lipid extract bilayers of Y2311 (A & C) and Y2310 (B & D) on silicon (111) supports.

These results however clearly illustrate that there is a difference in the effect of AmB on the lipid membranes based on the phenotype of the yeast and it sensitivity to the antifungal drug in vivo. The removal of ergosterol by AmB from the sensitive strain (Y2311) is consistent with the ergosterol-extracting AmB sponge-layer suggested recently as the underlying mechanism of AmB [1] and was also observed in *P. pastoris* membranes (8-02-717, 8-02-746 and 8-02-773). The AmB however inserts to a significant degree in the bilayer from the resistant *C. glabrata* strain Y2310, with marginal ergosterol extraction and some water penetration. This observation suggests the formation of pores, as seen in both model and bacterial membranes [2], proposed as the origin of AmB's toxic side effects. We can conclude that the yeast lipid bilayers from *C. glabrata* show promise as good candidates for the structural investigation of drug interaction with cell membranes, and we will continue the investigation by selective deuteration/reconstitution of different membrane components to elucidate the roles they play in the AmB mechanism.

References:

[1] de Ghellinck, et al., (2015). *BBA - Biomembranes*, *1848*(PA), 2317–2325.
[2] Venegas, B., et al., (2003). Biophysical Journal, 85(4), 2323–2332.