Tuberculosis related drug-lipid interactions demonstrated by evanescent field biosensor

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Introduction

Mycolic acids (MA), depicted in Fig.1, which are part of the cell envelope of *Mycobacterium tuberculosis*, play a major role in the pathogenesis of the bacteria (Dubnau *et al*., 2000; Fujita *et al*., 2007). The **objective** of this project was to find evidence, based on low molecular weight ligate/ligand interactions, for the mechanism of attraction between mycolic acids and cholesterol observed before (Benadie *et al.,* 2008). The cholesteroid nature of mycolic acids and its attraction of cholesterol may provide an important contribution to understanding how dormant TB survive in the host. The anti-fungal macrolide Amphotericin B is known to effect its biological activity by binding to cholesterol and related steroids (Baginski *et al*., 2002). The evanescent field biosensor is normally applied to measure protein-protein or protein-small ligand interactions. The ligand is sometimes immobilised into liposomes if it is a membrane embedded moiety or simply a hydrophobic entity. Here we demonstrate that small ligand /ligate interactions can also be measured with evanescent field biosensing, by proving that Amphotericin B binds to both cholesterol and MA.

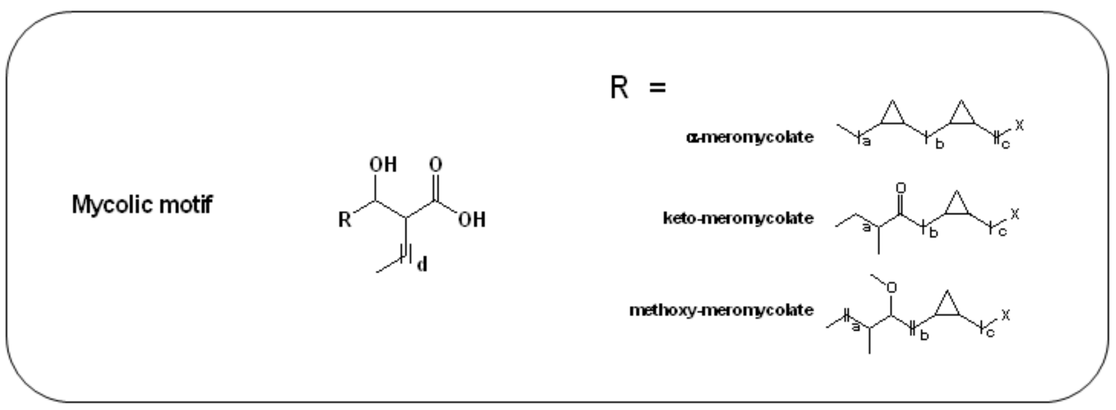

Fig.1 Generalised structures of MA from *Mycobacterium tuberculosis.* Hydrocarbon chain lengths are indicated as a, b or c, to a total of around 90 carbons per MA.

Experimental and Results

On an IAsys wave guide biosensor, the real time binding interactions were measured as depicted in Fig.2 (Thanyani *et al,*. 2008). As shown in Fig.3 the compound Amphotericin B associated with immobilized cholesterol liposomes. Similarly, Amphotericin B strongly associated with MA. In contrast, the interaction with both the synthetic protected α-MA and phosphatidyl choline (PC) liposomes indicated very little binding, proving the specificity of the AmB and MA/cholesterol interaction.

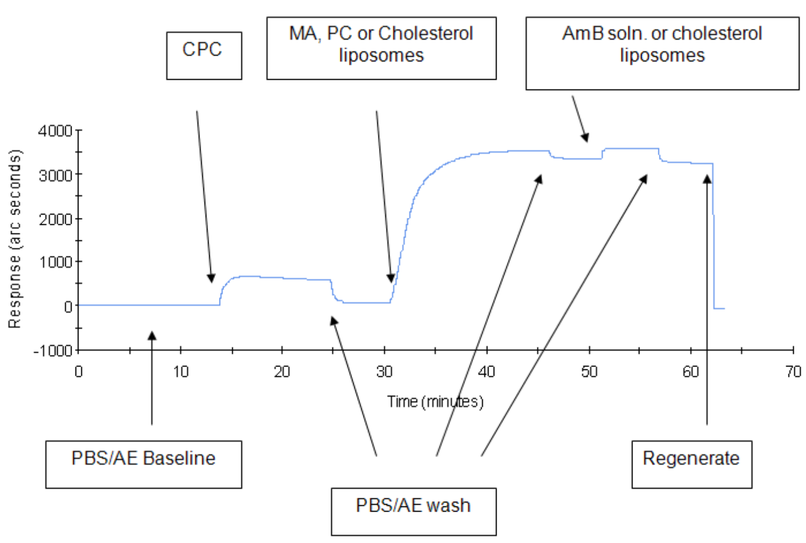

Fig.2 Sensorgram to determine the binding of soluble AmB (1 x 10^{-4} M) or cholesterol liposomes to either MA (MA:PC= 1:9) or cholesterol (Chol:PC= 3:6) liposome coats.

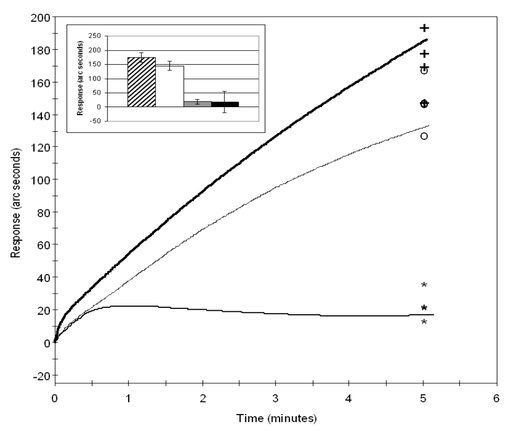

Fig.3 Binding of AmB to immobilized (a) MA, thick line, +, hatched bar; (b) cholesterol, thin line, o, white bar; (c) synthetic protected α-MA, dotted line, *, grey bar or (d) PC liposomes, black bar. Error bars indicate the standard deviation, n = 5 for each set.

The same principle was confirmed with the surface plasmon resonance ESPRIT biosensor where an octadecanethiol coated gold disk was mounted in the instrument and the individual liposomes immobilized without the CPC activation as described before (Fig.2). The binding interaction between Amphotericin B and either immobilized cholesterol-, MA- or 5-Bromomethylfluorescein (5-BMF)-MA liposomes were tested. The results (Fig.4) confirmed the ability of the ESPRIT biosensor to demonstrate that Amphotericin B recognizes both cholesterol and MA, as was previously shown with the IAsys biosensor. Here the specificity of interaction between AmB and MA was confirmed by showing weaker binding of AmB to MA after adding a bulky label to its carboxylic acid group.

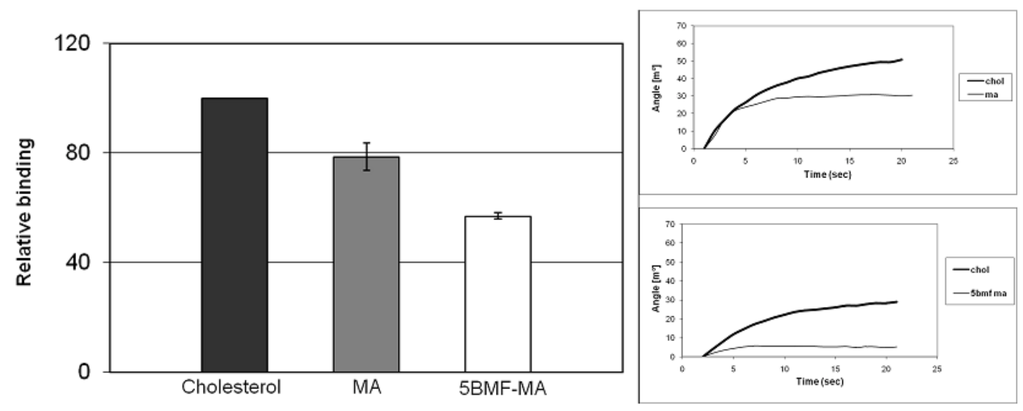

Fig.4 AmB binding to immobilized (a) MA, thin line, grey bar; (b) cholesterol, thick line, black bar and (c) 5BMF-MA, thin line, white bar. The error bars indicate the standard deviation, n = 3 for each set.

Discussion and conclusion

This is the first demonstration of the use of the evanescent field biosensor to show the interaction between low molecular weight ligate/ligand pairs. We demonstrated the interaction of Amphotericin B to either immobilized cholesterol or MA in liposomes using the evanescent field biosensor. The specificity of the interaction is confirmed by chemical modification of the mycolic acid with 5-BMF, which causes a significant decrease of binding with Amphotericin B. This suggests that MA may fold up to assume a cholesteroid nature. In its dormant state, TB bacilli are known to biofilm and secrete mycolic acids (Ojha *et al*., 2008). If these mycolic acids attract cholesterol, it may provide an explanation for the means whereby extracellular dormant mycobacteria in non-vascularised TB lesions in the lung can survive on a diet that mainly consists of cholesterol (Van der Geize 2007).

Acknowledgements

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