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Complex of lysozyme and Myramistin: formation and adsorption at the water–xylene interface

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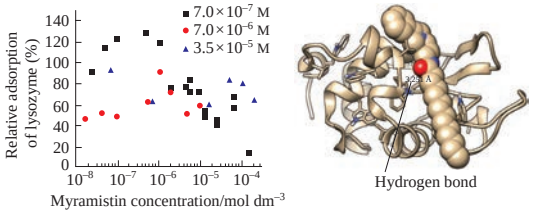
Lysozyme and Myramistin are able to associate when their molar ratio in solution reaches 1 : 1. Lysozyme preserves its secondary structure in the complex, whose surface activity at the water–*p*-xylene interface represents a model for the drug interaction with cellular membrane.



Keywords: lysozyme, Myramistin, coadsorption, liquid–liquid interface, protein–surfactant complex.

Benzyldimethyl[3-(tetradecanoylamino)propyl]azanium chloride commercially available as Myramistin or Miramistin represents a quaternary ammonium surfactant with antimicrobial properties, which is used as an anti-inflammatory and local adjuvant agent.[1–4] Its pharmacokinetics in relation to mucous membranes implies an interaction with mucus proteins such as lysozyme. In this work, an influence of Myramistin on lysozyme behaviour in the system of two immiscible liquids, namely water and *p*-xylene, has been investigated. This liquid–liquid interface can be considered as a model for cellular membrane.[5]

Content of the protein in the bulk of immiscible liquids system as well as at the liquid–liquid interface was measured using [^{3}H]lysozyme obtained by the tritium thermal activation method.[6] The experiment was carried out by the scintillation phase technique employing a scintillation cocktail based on *p*-xylene.[7,8] Hen egg white lysozyme was explored at concentrations of 7×10^{-7}, 7×10^{-6} and 3.5×10^{-5} mol dm^{-3} close to those found in human fluids like tears, blood serum or saliva,[9,10] while Myramistin was used at 5×10^{-8} to 5×10^{-4} mol dm^{-3}, and all the solutions were prepared in phosphate buffered saline (PBS). The lysozyme adsorption Γ was determined from radioactivity counting I for the two-phase system and the counting I_1 for an organic phase sample of known volume V_1, which was picked out and measured separately:

$$\Gamma = \frac{I - I_1(V/V_1)}{0.5\,\varepsilon\,a_{sp}\,S}, \quad (1)$$

where V represented total volume of *p*-xylene phase, ε was registration efficiency of tritium in the *p*-xylene phase, a_{sp} expressed the specific radioactivity of [^{3}H]lysozyme and S was an area of the liquid–liquid interface. The calculated lysozyme adsorption, normalized per its value for the free protein, *vs.* Myramistin concentration is shown in Figure 1.

For the lowest investigated lysozyme concentration of 7×10^{-7} mol dm^{-3} we found significant growth in the adsorption of protein with elevation of Myramistin concentration until the protein–ligand molar ratio reached

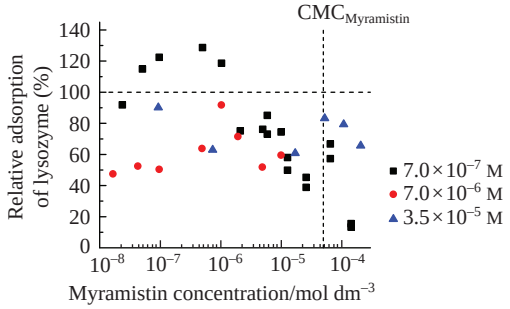


Figure 1 Relative adsorption of lysozyme at the water–*p*-xylene interface *vs.* Myramistin concentration. Vertical dashed line corresponds to the critical micelle concentration (CMC) of Myramistin equal to 5×10^{-5} mol dm^{-3}.

1 : 1, though further increase in the surfactant concentration reduced lysozyme adsorption at the liquid–liquid interface. In an experiment with 7×10^{-6} mol dm^{-3} protein, the displacement of lysozyme from the adsorption layer was observed, however this effect was less pronounced with elevation of the surfactant amount. The highest lysozyme content in the adsorption layer was reached at Myramistin concentration close to the one of protein, and further somewhat decreased. At lysozyme concentration of 3.5×10^{-5} mol dm^{-3} addition of surfactant reduced as well the amount of protein in the adsorption layer. However, at Myramistin concentration of *ca.* 5×10^{-5} mol dm^{-3} the highest lysozyme adsorption was observed. Note that this value of surfactant concentration corresponds to its CMC in PBS determined by tensiometry using the pendant drop technique. We assume that the close values of lysozyme and Myramistin concentrations at the maximum adsorption are associated with formation of a complex with most probable molar composition of 1 : 1 rather than represent simply a coincidence. The complex has higher ability to adsorb at the interface compared with the free protein. Further increase in the concentration of Myramistin reveals its stronger contribution

to displacement of lysozyme from the adsorption layer, thus the effect observed becomes less pronounced.

To determine the influence of Myramistin on the lysozyme secondary structure, circular dichroism (CD) spectra of the protein–surfactant mixture were recorded. Since chloride ion absorb strongly at wavelength less than 195 nm,[11] the measurements were carried out in buffer of the same pH value and the same phosphate concentration as in PBS, but lacking NaCl. CD spectra were obtained for lysozyme concentration of 1.0 g dm^{-3} (*i.e.*, 7×10^{-5} mol dm^{-3}). The amount of Myramistin was chosen to correspond to the protein–drug molar ratios of 1 : 1 and 1 : 10, *i.e.*, 7×10^{-5} and 7×10^{-6} mol dm^{-3}, respectively. The resulting CD spectra are shown in Figure 2.

It was found that the presence of Myramistin did not change the secondary structure of lysozyme. In all cases the far-UV CD spectra revealed two minima, namely at *ca.* 208 nm and *ca.* 224 nm, which confirmed α-helix-rich structure and thus indicated conformational stability of the protein in complex with the surfactant.[12,13] On the basis of these results an arrangement of Myramistin in its complex with lysozyme was suggested from molecular docking carried out using a Hex 8.0.0 protein docking software. The lysozyme structure with a PDB ID 6LYZ was employed for the calculations and the Myramistin structure was preoptimized utilizing an AMBER force field package. It was found that the complex was stabilized by hydrogen bond between Ala107 and the oxygen atom of Myramistin (Figure 3) as well as by tentative hydrophobic interactions between the surfactant molecule and hydrophobic residues of lysozyme such as Trp or Val, which are known to be included in its hydrophobic pocket.[14]

In summary, lysozyme can spontaneously form a complex with cationic surfactant with preservation of its structure up to the surfactant concentration equal to CMC. The complex has molar composition 1 : 1 and possesses surface activity. Competitive adsorption of free lysozyme, Myramistin and their complex leads to dependence of the protein adsorption on the surfactant concentration, which has a clear extremum. Formation of the complex between the protein and drug of the same charge

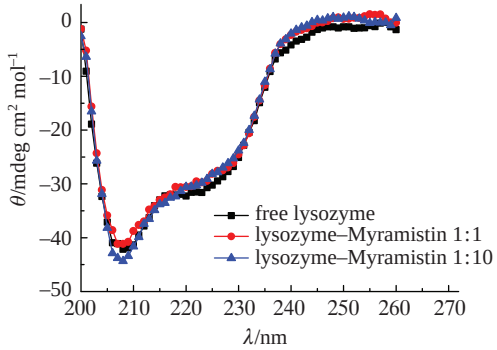


Figure 2 CD spectra in far-UV range for free lysozyme as well as lysozyme accompanied with equimolar amount and ten-fold excess of Myramistin.

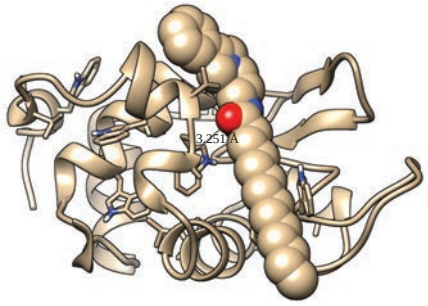


Figure 3 Structure of lysozyme–Myramistin complex. A hydrogen bond is indicated between Ala107 and the oxygen atom of the drug.

can influence enzymatic activity of lysozyme, which represents a goal for further research, while the results of this work may be taken into account for analysis of the Myramistin pharmacological effect.

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