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Electrochemical screening of self-assembling β-sheet peptides using supported phospholipid monolayers

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Abstract

In the context of the medical applications of β-sheet self-assembling peptides, it is important to be able to predict their activity at the biological membrane level. A study of the interaction of four systematically varied 11-residue (P11-1, P11-2, P11-6 and P11-7) and one 13-residue (P13-1) designed β-sheet self-assembling peptides with DOPC monolayers on a mercury electrode is reported in this paper. Experiments were carried out in 0.1 mol dm^{-3} KCl electrolyte with added phosphate buffer (0.001 mol dm^{-3}) at pH ∼ 7.6. The capacity–potential curves of the coated electrode in the presence and absence of the different peptides were measured using out-of-phase ac voltammetry. The frequency dependence of the complex impedance of the coated electrode surfaces in the presence and absence of the peptides was estimated between 65,000 and 0.1 Hz at −0.4 V versus Ag/AgCl 3.5 mol $^{-3}$ dm^{-3} KCl. The monolayer permeabilising properties of the peptides were studied by following the reduction of Tl(I) to Tl(Hg) at the coated electrode. Of the five peptides studied, P11-2, P11-7 and P13-1 interact most strongly with the DOPC layer. P11-1 which has a polar primary structure shows no obvious interaction with the phospholipid but surprisingly, it permeabilises the phospholipid layer to Tl^+.

Keywords: Phospholipid monolayers; Self-assembling beta-sheet peptides; Capacitance; Tl(I) permeability; Tryptophan; Screening

1. Introduction

The interaction of biologically active peptides with biological membranes in particular the phospholipid component has received much interest in the last decade [1–12]. The results have relevance to biological mechanisms since peptide and protein interactions with biological membranes are of great significance in many aspects of physiology [7], such as cell signalling and toxicology. Of particular interest are the antimicrobial peptides [1,2,5,6,9–12] and the membrane-active peptides [3], which act by disrupting biological membrane structure and function. The ability of antimicrobial peptides to kill bacteria while not disrupting native cells is attracting a great deal of attention, especially since traditional antibiotics are becoming increasingly difficult to produce and because of increased bacterial mutation. The mechanism of action of antimicrobial peptides is not entirely understood, but it is clear that they interact not with membrane proteins, but with the lipid matrix itself, and therefore leave little or no possibility for mutation which could affect their performance.

Three models of membrane rupturing mechanisms have been proposed to date: barrel-stave [13–17], carpet [18,19], and toroidal [20–22]. According to the barrel-stave model, peptides bound to the membrane recognize each other and oligomerize. Upon oligomerization, antimicrobial peptides orient themselves, allowing the hydrophobic surface to interact with the hydrophobic core of the membrane and the hydrophilic surface to point inward to create a hydrophilic transmembrane pore. The carpet model suggests that antimicrobial peptides initially bind to and cover the surface of the target membrane. The electrostatic interaction between the peptide and the lipid head group imposes strain in the

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membrane, and membrane permeation is induced only at sites where local peptide concentration is higher than certain threshold values. In the toroidal model, peptides similarly bind and interact with lipid head groups, imposing a positive curvature strain on the membrane (e.g., magainin 2) and producing channels where the polar headgroup region expands to form "toroidal" pores. In these mechanisms, the interaction between the phospholipid component of the membrane and the peptide is often of particular importance [23,24]. Because of this phospholipid–peptide interactions are the subject of considerable interest.

Several molecular properties of the peptides are known to promote interaction with the phospholipid such as hydrophobicity [25], number of residues [26] and the presence of specific peptide residues such as tryptophan [26].

In order to study the interaction of peptides with biological membranes, biological membrane models are often used [27]. These represent a significant approach since the experimental conditions can be rigorously controlled and selective aspects of the interaction can be investigated. Membrane models used to study phospholipid–peptide interaction have ranged from free-standing bilayers [27] to monolayers at the air–water [28] interface to supported monolayers and bilayers [29]. One of the most powerful supported membrane models available is that of a phospholipid monolayer/bilayer on a mercury electrode [30]. The great advantage is its inherent reproducibility and ease of use and the ability to control the potential and measure the current very precisely. The system has acted as a good host for the gramicidin monomolecular channel so that channel function can be monitored using the Tl^+/Tl(Hg) system as a redox probe [31]. At the same time interactions with the phospholipid monolayer can be investigated since the pure monolayer system is virtually defect-free and self-sealing. Any modification to the structure can be easily monitored. Electrochemical methods of impedance are a very sensitive way to monitor the structure and properties of the layer and a novel impedance model has been developed using the system to test it [32]. Recently an investigation of the interaction of gramicidin peptide derivatives with the monolayer was carried out using voltammetric and impedance techniques [33]. Results were encouraging and they correlated well with those of independent experiments carried out with membrane models of monolayers at the air–water interface. This has provided the instigation to use the system to study interactions with other peptides. In order to isolate the molecular principles associated with the interaction it was deemed appropriate to use custom designed peptides. In this case the type of phospholipid–peptide interaction could be related to specific molecular characteristics.

This paper reports on the interaction of custom designed peptides with phospholipid monolayers of dioleoyl phosphatidylcholine (DOPC) on mercury. The particular techniques used were: (i) ac voltammetry to examine the effect of peptide interaction on the phospholipid phase transitions in capacitance–potential curves, (ii) ac impedance to look at the extent of penetration of peptide into the monolayer and any modifying effect on the monolayer structure from impedance–frequency data, and (iii) sampled-current voltammetry of the Tl^+/Tl(Hg) redox probe to look for permeabilising effects of the peptide.

In recent years, the biological β-sheet motif has been exploited to design simple *de novo* peptides that self-assemble in a hierarchical manner to form a variety of well-defined twisted elongated nanostructures [34–37] such as tapes (single molecule in thickness), ribbons (a pair of stacked tapes back to back), fibrils (a bundle of stacked ribbons) and fibres (a pair of fibrils interacting edge-to-edge). A theoretical model has been developed to rationalise this self-assembly process [38,39]. At concentrations typically higher than 0.5% (v/v) in solution these micrometer-long aggregates can form isotropic solid-like organogels and hydrogels, as well as nematic liquid crystalline fluids and gels. The self-assembly can be switched on or off by a variety of external chemical triggers such as pH and ionic strength [40,41]. On the one hand these peptides provide an ideal model system for the investigation of the principles that drive biomolecular peptide self-assembly, and in particular the formation and stabilisation of elongated β-sheet structures, e.g. present in amyloid diseases. On the other hand the mechanical, structural and bioactive properties as well as the surface chemistry of these systems can be finely controlled by appropriate peptide design. Therefore, there is the opportunity to design self-assembling peptides with a combination of properties appropriate for specific applications, e.g. in the biomedical field such as scaffolds for tissue engineering, or biomaterials for personal care and dental hygiene. In the light of these applications, it was considered appropriate to test the putative membrane-activity of these β-self-assembling peptides using the DOPC coated electrode system.

The structures of the peptides tested in this study are shown in Scheme 1.

2. Experimental

2.1. Apparatus and materials

Two distinct measurements were carried out using the electrochemical apparatus. The first series of measurements focused on impedance measurements in which no faradaic process is involved. These experiments concentrated on capacitive elements [30,32,33]. The second series investigated the transport of Tl^+ ions in which a faradaic process is involved [31]. The rationale for using Tl^+ as a probe is the following. (1) Tl^+ is isoelectronic with K^+ and thus it is an effective probe for this ion's behaviour and the alkali metal ions in general. (2) Tl^+ undergoes a rapid reversible redox reaction on the mercury surface with a reduction potential at about −0.42 V versus Ag/AgCl, 3.5 mol dm^{-3} KCl which is in the potential domain of the low capacity and ion impermeable region of the DOPC monolayer. As a consequence