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Bioelectrochemistry 55 (2002) 5–7

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Single channel recordings of α-hemolysin reconstituted in S-layer-supported lipid bilayers

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Received 1 June 2001; accepted 29 June 2001

Abstract

Previous studies demonstrated that lipid membranes attached to a proteinaceous crystalline surface-layer (S-layer) revealed a prolonged lifetime and showed a reduced tendency to rupture in the presence of membrane active molecules. In addition, comparative studies on folded and S-layer-supported lipid membranes (SsLM) revealed an uniform capacitance of 0.64 ± 0.04 μF/cm^2 for both composite membranes. In the present study, the feasibility to reconstitute the channel-forming protein α-hemolysin (αHL) into SsLM at single channel resolution was investigated. Single αHL channels could be recorded and the intrinsic properties like unitary conductance, current–voltage characteristics, and closure was found to be similar at both membranes. Thus, the tightly attached S-layer allowed complete reconstitution of αHL channels in SsLM.

Keywords: Crystalline bacterial surface layer (S-layer); Supported lipid membranes; Planar lipid bilayer; α-hemolysin; Reconstitution; Single channel

1. Introduction

Crystalline monomolecular bacterial cell surface layers (S-layers) represent the outermost cell envelope component of organisms of almost every taxonomic group of walled bacteria and archaea [1,2]. S-layers are isoporous structures, composed of a single protein or glycoprotein species. Isolated S-layer subunits are able to assemble into monomolecular arrays at many interfaces and particularly on lipid films, mimicking the supramolecular architecture of archaeal cell envelopes. [2,3]. Certain protein domains of the S-layer protein interact with some headgroups of the adjacent lipid layer, resulting in a lipid bilayer membrane with an enhanced stability [4,5]. Interestingly, the tight lipid–protein interaction has minute impact on the hydrophobic part of the lipid membrane as determined by X-ray [6] and capacitance measurements [4]. These intrinsic features make the composite S-layer/lipid membranes attractive for the use at biosensors based on transmembrane channel proteins as sensing elements. The aim of the present work was to study the feasibility of single channel measurements in S-layer-supported lipid membranes (SsLM). For this purpose, the staphylococcal toxin α-hemolysin (αHL) has been reconstituted in SsLM (Fig. 1).

2. Experimental

Growth, cell wall preparations, and extraction of the S-layer protein SbpA from *Bacillus sphaericus* CCM 2177 (Czech Collection of Microorganisms) were performed as described elsewhere [7]. For the formation of SsLM, a lipid membrane was generated as described below and after half an hour, 0.5 ml of a SbpA solution (1.8 mg protein/ml) was injected into the *trans* chamber. The recrystallization was completed after 3 h as determined by transmission electron microscopy as described previously [4]. Folded planar lipid bilayers were formed from diphytanoyl phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) and hexadecylamine (Fluka, Buchs, Switzerland) at a molar ratio of 10:3 on a 130-μm diameter orifice in a Teflon septum that separated the *cis* and *trans* chambers [4,8]. The protein α-hemolysin from *Staphylococcus aureus* (αHL, Wood strain 46, American Type Culture Collection) was a kind gift of H. Bayley of the A&M University, Texas, and purified as described elsewhere [9]. Single channels were formed by adding < 2.5

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1567-5394/02/$ - see front matter
PII: S1567-5394(01)00148-7

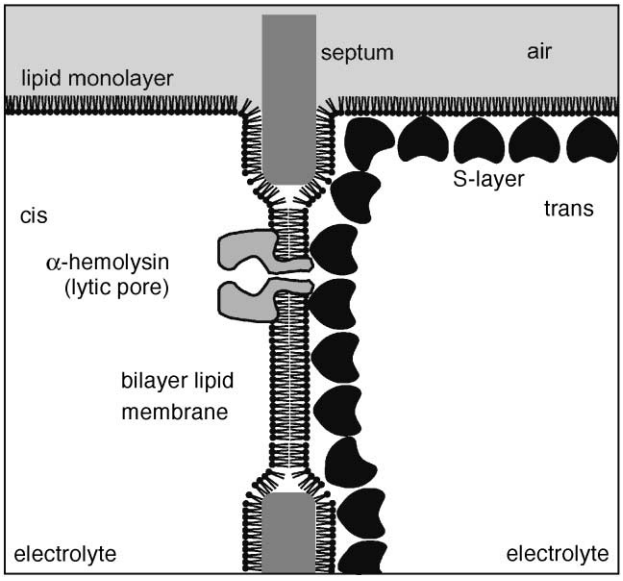

Fig. 1. Schematic illustration of the assembly of α-hemolysin oligomers in an S-layer-supported bilayer membrane (not drawn to scale).

μg of αHL monomers to 4 ml of buffer in the *cis* chamber. All experiments were performed in 100 mM KCl, 2 mM $CaCl_2$, pH 7.4 at 22 ± 1 °C. The current flow through the αHL channel was measured by an EPC 9 patch clamp amplifier (HEKA Elektronik, Lambrecht, Germany). The settings of the two built-in Bessel filters were 10 and 1.5 kHz, respectively.

3. Results and discussion

Electron microscopical studies demonstrated the recrystallization of SbpA into large continuous lattices on the planar lipid bilayer. The attachment of the proteinaceous lattice caused no significant change in the specific capacitance of the lipid membrane, which was 0.64 ± 0.04 μF/cm^2 for both the folded and the SsLM. Thus, the attached S-layer obviously has no impact on the area, thickness, or the dielectric properties of the hydrophobic part of the membrane. The capacitance is also close to data reported for common folded lipid bilayers [10]. The benefit in the use of SsLM is the enhanced mechanical stability as SsLM revealed a prolonged lifetime and showed a decreased tendency to rupture in the presence of a high amount of αHL [4]. In a previous study, a clear difference in the reconstitution behaviour of αHL channels was found depending on the side of the SsLM to which αHL monomers have been added [4]. Staphylococcal αHL formed lytic channels when added to the lipid-exposed side. The assembly was slow compared to unsupported membranes, perhaps due to an altered fluidity of the lipid bilayer. No assembly could be detected upon adding αHL monomers to the S-layer-faced side of the composite membrane. Therefore, the intrinsic molecular sieving properties of the S-layer lattice do not allow passage of αHL monomers through the S-layer pores to the lipid bilayer.

In the present paper, the feasibility to perform single channel measurements was investigated by using the transmembrane protein αHL (Fig. 1). The water-soluble monomer has a high affinity to lipid membranes and once seven attached individual monomers converge, the thin strand of amino acids in the center of each subunit borrows into the lipid membrane to form the channel. Indeed, as in unsupported folded membranes, single αHL channels reconstituted also in SsLM. The conductance of single channels was determined by continuously incorporating channels (Fig. 2A). The unitary αHL channel conductance was calculated to 72 ± 3 pS at ≤ +60 mV and 61 ± 1 pS at ≤ − 60 mV. At a potential higher than ± 60 mV, no linear increase of the transmembrane current was observed (Fig. 2B) and the calculated unitary conductance even decreased with increasing potential. The same behaviour was found with unsupported membranes and is also in accordance with previously reported data [11]. Another feature observed with folded and SsLM was the closure of single αHL channels (data not shown). These closing events are most probably due to the presence of calcium ions that are known to show voltage-dependent blocking effects on αHL [12]. All the properties of single αHL channels are similar in both systems, indicating that the tightly attached

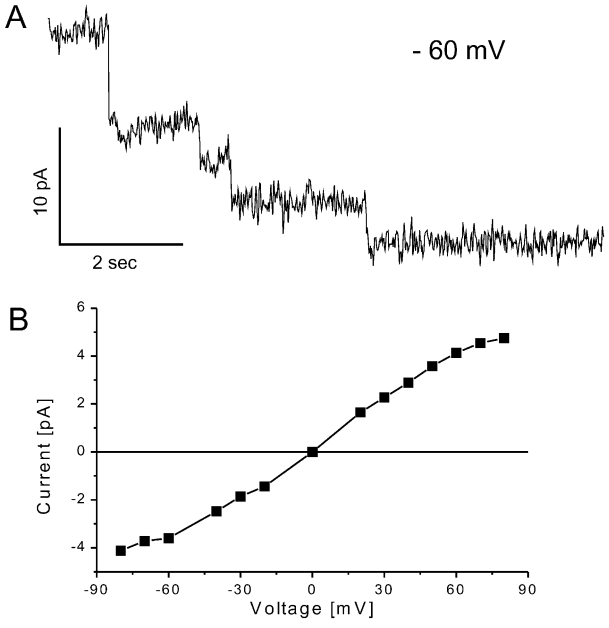

Fig. 2. (A) Current steps of reconstituted α-hemolysin (αHL) channels in an S-layer-supported lipid membrane (SsLM) clamped at − 60 mV. Each current step (except the first one) is due to the opening of a new ionic channel into the membrane. (B) Current–voltage characteristics of αHL channels reconstituted in an SsLM. The curve has been normalized to the mean current flowing through a single channel. The electrolyte solution consisted of 0.1 M KCl and 2 mM $CaCl_2$, pH 7.4.

porous S-layer provided far enough space for the stem region of the αHL cannel and allowed full and undisturbed penetration. In contrast, the addition of αHL to supported bilayer membranes comprised of alkanethiol and phospholipids on a gold surface resulted only in an incomplete reconstitution and no single channel formation could be detected [13]. In this context, it is also interesting to note that bilayer lipid membranes have also been generated on smooth S-layer lattices deposited on a microfiltration membrane [10]. This membranes are called SUM-supported lipid membranes [3,10]. In accordance with SsLM, SUM-supported lipid membranes also showed complete reconstitution of αHL channels. Most interestingly, without the S-layer as a separating structure between the microfiltration membrane and the lipid membrane, no channel formation of αHL could be observed. Thus, the S-layer provides a biomimetic environment for domains of transmembrane proteins protruding from the lipid membrane.

4. Conclusion

The present results demonstrated the feasibility to use SsLM for the investigation of unitary αHL channels. Since SsLM reveal a decreased tendency to rupture [4], these composite structures represent an appealing biomimetic system for studying structural and functional properties of membrane proteins. In addition, a great variety of solid surfaces can be completely covered with a closed ultrathin S-layer [2]. This water-containing layer, separating the lipid membrane and reconstituted proteins from inorganic surfaces, will allow the combination of relevant biological interactions as sensing elements with electronic devices as read-out systems. For example, αHL assembled at SsLM might lead to new nanotechnological applications, particularly in ultrarapid sequencing of nucleic acids [14,15] and in sensor technology [16,17].

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