Biochemical and Biophysical Research Communications 427 (2012) 385–391

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

A high resolution electro-optical approach for investigating transition of soluble proteins to integral membrane proteins probed by colicin A

Alf Honigmann [a,1], Lakshmi Padmavathi Pulagam [b,2], Michael Sippach [a,b], Philipp Bartsch [a], Heinz-Jürgen Steinhoff [b], Richard Wagner [a,*]

[a] Universität Osnabrück, FB Biologie/Chemie, Barbara Str. 13, 49076 Osnabrück, Germany
[b] Universität Osnabrück, FB Physik, Barbara Str. 7, 49076 Osnabrück, Germany

ARTICLE INFO

Article history:
Received 6 September 2012
Available online 18 September 2012

Keywords:
Membrane
Artificial bilayer
Fluorescence
Electro-optical recording
Colicin A
Colicin A-oligomerization
Membran-Protein insertion

ABSTRACT

The transition from water soluble state to an integral membrane protein state is a crucial step in the formation of the active form of many pore-forming or receptor proteins. Albeit this, high resolution techniques which allow assay of protein membrane binding and concomitant development of the final active form in the membrane await further development. Here, we describe a horizontal artificial bilayers setup allowing for simultaneous electrical and optical measurements at a single molecule level. We use the membrane binding and subsequent channel formation of colicin A (ColA) a water soluble bacteriocin secreted by some strains of *Escherichia coli* to demonstrate the potential of the combined electro-optical technique. Our results expand the knowledge on ColA molecular details which show that active ColA is monomeric; membrane binding is pH but not membrane-potential ($\Delta\phi$) dependent. ColA is at $\Delta\phi$=0 permeable for molecules $\geqslant$1 nm. Although ColA exhibits low ion conductance it facilitates permeation of large molecules. Our electro-optical recordings reveal ColA monomeric state and the chimeric character of its pore.

1. Introduction

Pore-forming toxins are secreted by many pathological bacteria as a weapon against host cells or against competing bacteria [1,2]. The mechanism of action generally involves the secretion of the monomeric toxin into a water soluble conformation. Upon contact of the toxin with the target cell-membrane a conformational change is triggered which induces membrane insertion and in most cases an oligomerization process. The toxic action of colicins is either nuclease activity in the cytosol or ion-channel formation in the plasma membrane of the target *Escherichia coli* cells. The crystal structures of the water-soluble conformation revealed that all pore-forming colicins are arranged in three domains [3,4]. The domains were named according to their specific function: the receptor binding domain, the translocation domain and the channel-forming domain [5,6]. At the plasma membrane the pore-forming domain inserts into the bilayer and forms a voltage-dependent ion channel which short circuits the vital electro-chemical gradient of the target cell [7,8]. The high resolution structure of the water soluble conformation of the pore-forming domain of ColA shows a ten-helix bundle containing a central hydrophobic helical hairpin in solution [3]. The structural similarities between colicins and other pore forming toxins suggest that a conserved mechanism of pore-formation is at work.

Contradicting properties of the open channel have greatly complicated the establishment of an appropriate structural model: even though the ion conductivity of the open conformation is very low, but nevertheless large ions or even small folded proteins can be translocated by ColE1 and Col-Ia [9–11]. Different studies point to a monomeric pore [6,12,13], but also to oligomeric pores of Col IA in lipid bilayers [14].

Here we used an electro-optical approach to characterize the membrane binding of ColA and the subsequent channel opening upon application of an electrical field. Our electro-optical setup is comprised of a horizontal lipid bilayer chip, representing a modified version of the planar lipid bilayer technique and a 3D-confocal laser scanning microscope equipped with single photon detection unit. This setup allows single channel conductance- (voltage clamp), diffusion- (FCS), environment- (fluorescence lifetime) and oligomerization- (FRET, FIDA) measurements, simultaneously. Accessing these parameters simultaneously is promising in the context of ColA because to study incorporation into the membrane

* Corresponding author. Address: Biophysics, Dep. Biology/Chemistry, University Osnabrueck, Barbarastr. 13, 49076 Osnabrueck, Germany. Fax: +49 541 969 2243.
E-mail address: wagner@uos.de (R. Wagner).
[1] Present address: Max-Planck-Institut für Biophysikalische Chemie, Abteilung NanoBiophotonik, Am Faßberg 11, 37077 Göttingen, Germany.
[2] Present address: Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, USA.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.09.069

and the open channel conformation it is required to have a controllable and stable membrane potential which was lacking in previous studies on colicins.

2. Materials and methods

2.1. Chemicals

E. coli polar lipid extract was purchased from Avanti Polar Lipids (Alabaster, AL). *N*,*N*-dimethyl-*N*-(iodoacetyl)-*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) ethylenediame (NBD amide) was purchased from Molecular Probes (Eugene, OR). The organic dyes Atto488-maleimide and Atto647N-maleimide were purchased from Atto-Tec (Siegen, Germany). Lipids were stored in methanol/chloroform (1:1) under nitrogen at −20 °C.

2.2. Mutagenesis, preparation and purification of ColA

The plasmid pLR1, which encodes the wild-type ColA, was used as template to replace different residues within the ColA sequence with a cystein. Mutagenesis, activity test of mutant strains and preparation of the different Cys-mutant proteins was performed essentially as described [15].

2.3. Fluorescence labeling of single cysteine ColA mutants

ColA-NBD and $ColA_x$-488 and $ColA_x$-647N were produced as described [15]. The products ColA-F were separated from non-reacted dyes by size exclusion chromatography using NAP-5 columns (GE Healthcare, Piscataway, NJ).

The degree of labeling (DOL) was determined by absorption spectroscopy. Protein concentration was estimated from A_{280} (corrected by the dye absorption at 280 nm) with a calculated ε of 51,910 M^{-1} cm^{-1} (Expasy ProtParam). The resulted DOLs varied between 0.6 and 0.9.

2.4. Horizontal lipid bilayers and electrophysiology

The construction and handling of the horizontal bilayer chip (HLB) was as described in detail [16]. Bilayer formation was monitored optically and electrically. Fluorescence fluctuation analysis and electrical measurements were performed essentially as described [16].

2.5. Calcein efflux from liposomes incubated with ColA

Calcein efflux from liposomes incubated with ColA was monitored as described in the Supplements.

2.6. Confocal microscopy setup

Confocal imaging and fluorescence fluctuation recordings and analysis were performed on a modified Insight Cell 3D microscope from Evotec technologies (Hamburg, Germany, now Perkin Elmer), as described in detail [16].

2.7. Steady state fluorescence measurements

Steady state fluorescence measurements were performed with a Jasco FP 6500 fluorimeter (Jasco, Gross-Umstadt, Germany) as describe in Supplemental Fig. S2.

3. Results

3.1. Membrane binding of ColA is pH dependent

We studied the *in vitro* binding of ColA to membranes made of natural *E. coli* lipids using horizontal lipid bilayers as well as large uni-lamellar vesicles (LUV). ColA was labeled either with the fluorescent dye NBD to probe a change in hydrophobicity upon membrane binding or with Atto488, 647 to measure the diffusion properties in aqueous solution and at the bilayer. Fig. 1A shows a typical bilayer from *E. coli* lipids, the membrane was imaged by scanning the XZ plane. Without fluorescent label the bilayer is not visible on the image (Fig. 1A and B). Only the auto-fluorescence of the PTFE foil and the lipid torus are observable. When ColA166-488 88 was added to the trans compartment of the bilayer chip ($C \approx 10$ nM) the fluorescence of ColA166-488 88 is clearly visible on the trans side of the bilayer. The bilayer is indirectly visible, since it acts as a diffusion barrier for ColA166-488 88. Binding to the bilayer was studied at pH 7 (Fig. 1A) and pH 4 (Fig. 1B), respectively. At pH 7 no significant accumulation of $ColA_{166}$-488 at the bilayer was detectable (Fig. 1A), while at pH 4 binding to the bilayer was detected directly after addition of $ColA_{166}$-488 (Fig. 1B). The bright spots in the trans solution indicate that the protein in solution tended to aggregate at low pH (Fig. 1B).

A holding potential of +100 mV (positive on the side of ColA addition) did not significantly increase ColA166-488 88 binding (data not shown). We therefore can conclude that an applied membrane potential does not affect membrane binding of ColA.

3.2. Electrophysiological properties of single ColA channels

The concentration of ColA added to the bilayer was adjusted to result either in single or multi-channel activities that were dependent on pH (see Figs. 1 and 2). For the analysis of the single channel conductance of ColA a trans-membrane potential of 80–100 mV was applied to induce the open channel conformation. The typical single channel current trace at pH 7 (Fig. 2A) reveals gating of the ColA channel into several conductance states. The mean single channel conductance at different membrane potentials (Fig. 3B) revealed conductance histograms with peak values of $G_{max} = 10ps$ at pH 7 and $G_{max} = 10ps$ at pH 4, (1 M KCl) respectively. Interestingly, the mean single channel conductance at pH 4 was significantly lower (≈ 2-fold) compared to pH 7,which could be an indication for a reduced pore diameter and/or changes in the electrostatics of the channel pore due to protonation of amino acid residues at pH 4. As observed for other members of the colicin family [9] the single channel conductance of ColA is even lower than the one of the narrow gramicidin A channel [17].Using the approximation of a water filled cylindrical pore [19] with a restriction zone of 2 nm, the low conductance would imply a diameter of $d_{ch} = 1.6$ Å of the ColA channel (see Supplement). The strength of membrane potential necessary to open the channel (see Fig. 2D) displays a steep exponential dependence on the membrane potential with $v_p = 0.5 \approx 90mv$. Hence, the membrane potential values for efficient open channel formation (+90 mV) determined *in vitro* would be sufficient for channel incorporation with $0.5 < p_{open} < 1$ in the *E.coli* plasma membrane *in vivo* with reported values of $V_m \cong +85$ mV, pH 5 and $V_m \cong +145$ mV, pH 8, as well [18].

The dependence of open? channel formation on the electrical field was tested with an ensemble of channels incorporated in the bilayer. A trans-membrane potential of 80 mV was applied for 15 s, which resulted in a linear increase of current due to steady/successive? opening of ColA channels (Fig. 2C). When the direction of the applied potential was abruptly inverted, the number of open channels decreased exponentially with a time constants of

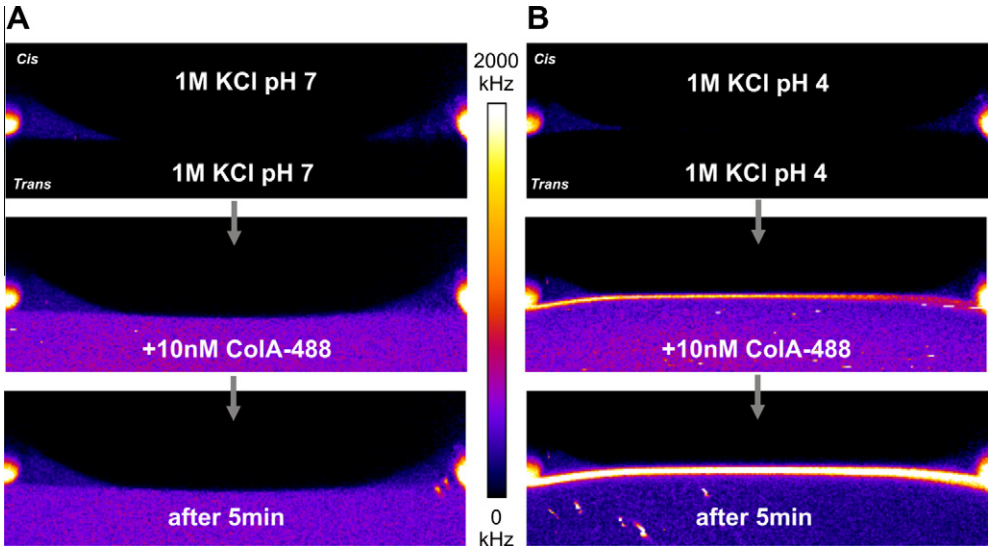

Fig. 1. pH dependent binding of ColA to lipid membranes. (A) Horizontal lipid bilayers prepared in 1 M KCl pH 7 buffer. A single cystein mutation introduced at position 166 of ColA (loop between helix 8 and 9, see Supplemental Fig. 1) was labeled. After addition of 10 nM fluorescently labeled ColA166-488 to the trans compartment, the lipid bilayer was indirectly visible as the barrier between cis and trans. (B) Horizontal lipid bilayers prepared in 1 M KCl pH 4 buffer. At pH 4 the same amount of ColA166-488 added to the trans side resulted in a distinct membrane staining.

τ = 11.6 s to zero. These results show that ColA channel opening is strictly dependent on the orientation of a trans-membrane potential. To open ColA channels the trans-membrane electric field has to be positive on the side of ColA binding, as it is the case *in vivo*. Channel opening *in vitro* is reversed by inverting the membrane potential. This strict dependence also shows that incorporation of the Col A channels occurred unidirectionally.

3.3. ColA induces calcein efflux from liposomes at acidic pH

In order to gain further information on the size of the ColA channel vestibules a fluorescence assay based on self-quenching was used to probe the translocation of the bulky dye calcein through ColA pores formed in liposomes (Fig. 2E). Liposomes containing a self-quenching concentration ($\approx$ 100 mM) of calcein were prepared by sonication and subsequent size exclusion purification. The pH inside the liposomes was either adjusted to pH 7 or pH 4. Calcein containing liposomes were added to an excess volume (1:200) of buffer adjusted to pH 7 or pH 4. Four pH permutations of inside and outside pH of 4 and 7 were produced (see Fig. 2E and legend). After equilibration (200 s), ColA was added to the liposomes (Fig. 2F). For two of the four pH configurations a rapid increase of fluorescence emission was detected upon addition of ColA, indicating that calcein is rapidly released from the liposomes. Efflux was only observed with outside pH 4. With outside pH 7 no calcein release was detected. These results indicate that the calcein efflux is specifically mediated by the ColA channel. These results are startling in two ways. In the first place, ColA should not be able to form open ion channels in liposomes without applied membrane potential at all (see Fig. 2D). Secondly, the calcein-efflux through ColA seemingly conflicts with the single channel ion conductance since it requires the diameter of the open channel has to exceed 1 nm. (calcein has a molecular size of about a = 9.5 Å b = 13 Å c = 13,4 Å (see Supplement).

3.4. Oligomerization of ColA

All differently labeled $ColA_X$-mutants displayed ion channel activity in planar bilayers like shown in Fig. 2A–D and are thus considered functional (details not shown). To study possible oligomerization a FRET pair of ColA monomers was prepared by labeling single cystein mutants with a donor dye (ColA166-488 88) and an acceptor dye ($ColA_{166}$-647). In steady state FRET measurements using soluble and liposome reconstituted mixtures of ColA166-488 88 and $ColA_{166}$-647 (see Supplemental Fig. S2) we could not detect any FRET, indicating that in solution and in liposomes no oligomerization occurred regardless in which positions the labels on the monomers were arranged (see Fig. S1A).

We then performed in a horizontal lipid bilayer setup confocal fluorescence lifetime measurements with the same $ColA_{166}$ FRET pairs described as above. In practice the determination of the donor lifetime is the most reliable readout to detect FRET because the fluorescence lifetime can be measured independent of concentration effects with high precision.

The laser focus was placed in the trans solution and the donor lifetime was determined in the presence and absence of the acceptor. ColA was then allowed to bind to the bilayer for five minutes. Finally, the excess protein was washed out by perfusion of the trans compartment. The laser focus was then placed directly on the bilayer containing the bound ColA and the donor lifetime was determined again (Fig. 3D). Secondly FCS-data were recorded to estimate the binding of ColA to the bilayer (Fig. 3C).

The lateral diffusion time of the donor $ColA_{166}$-488 was $D_{lat} \approx 50\ \mu m^2/s$ in the trans solution. This D_{lat} value indicates that the soluble form of the ColA has an asymmetric shape which can be best approximated by a prolate of revolution with a axial ratio of ρ = a/b = 12.9 (see calculation 2 Supplement). This hydrodynamic shape is a reasonable approximation of the shape deducible from the crystal structure of Col Ia (4). After accumulation of $ColA_{166}$-488 at the bilayer the diffusion time decreased significantly to $D_{lat} \approx 5\ \mu m^2/s$, which indicates stable binding of the toxin to the bilayer. For comparison, the lateral diffusion time of a fluorescently labeled lipid in the horizontal bilayer under the same conditions was determined to be $D_{lat} \approx 10\ \mu m^2/s$ (26). By comparing these two D_{lat} values and considering that the cross-sectional area of the used lipid molecules is in the order of $\varnothing \approx 35$ Å (34) one can infer that only minor parts of ColA can be completely immersed in the membrane. The fluorescence lifetime of the donor $ColA_{166}$-488 in solution was 3.6 ns. The lifetime of $ColA_{166}$-488 did not change significantly after the protein was bound to the

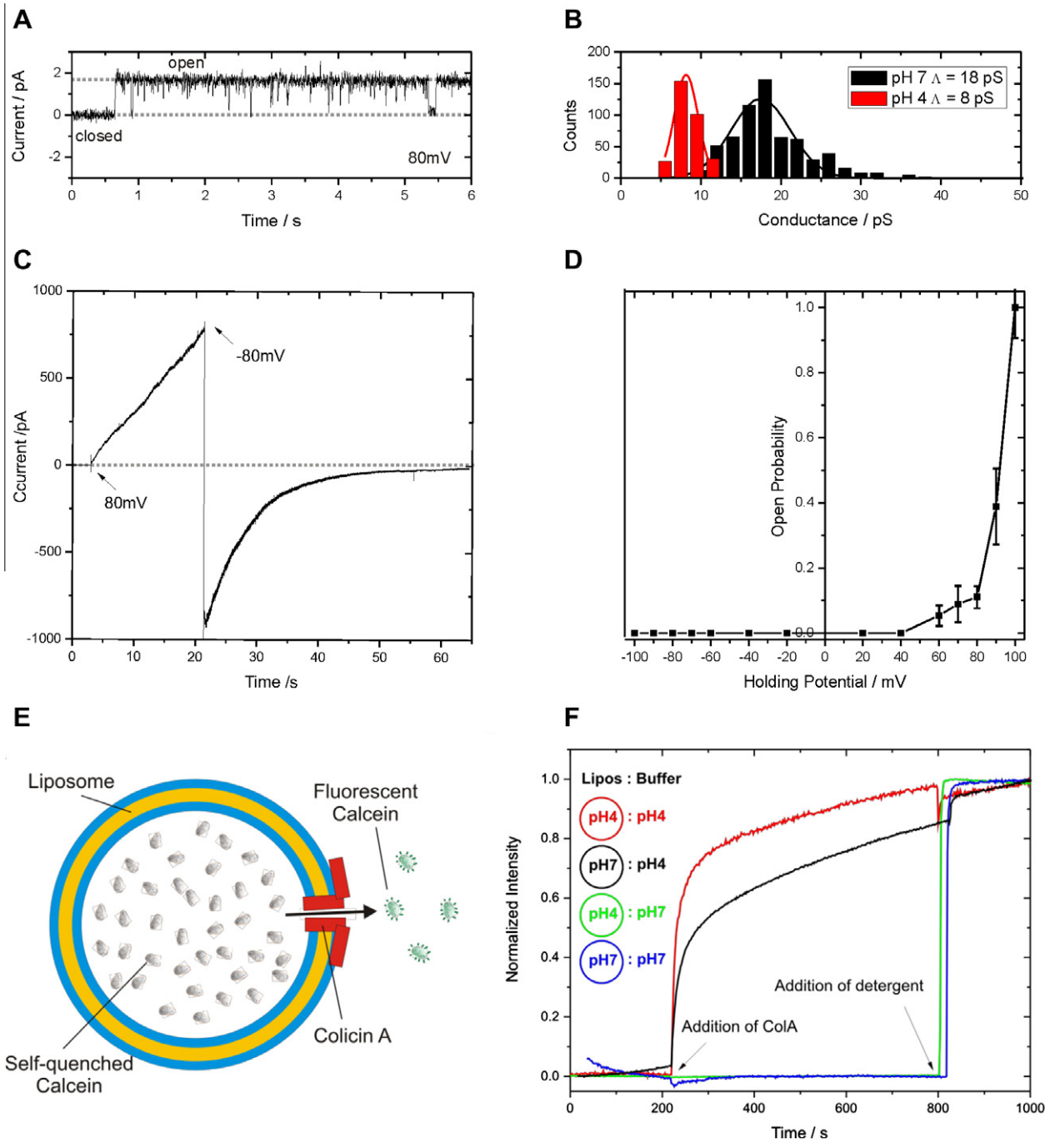

Fig. 2. Electrophysiological characteristics of ColA in lipid bilayers; calcein release from liposomes incubated with ColA. (A) Single channel recording of ColA at V_m = +80 mV in 1 M KCl pH 7. (B) Conductance histogram derived from more than 500 gating events showing a narrow distribution with a mean conductance of 18 pS at pH 7 and 8 pS at pH 4. (C) Multi-channel recording of ColA at V_m = +80 mV (ColA addition side) in 1 M KCl pH 7. (D) Open probability of ColA at different holding potentials. The open channel conformation was populated at potentials >60 mV. Efficient opening was found at V_m > 90 mV. (E) A schematic representation of the experimental design is depicted. Calcein is trapped in a self-quenching concentration of >80 mM inside LUVs. When calcein can permeate through open ColA channels the fluorescence emission will increase. (F) Time course measurements of liposome-trapped calcein using four different pH configurations. ColA was added to the cuvette after 200 s. In case of pH 4 in the outside solution the fluorescence increased rapidly after ColA addition, which indicates that calcein was released from the liposomes (red and black trace). In case of pH 7 in the outside solution no increase was observed (green and blue trace). All calcein was released from the liposomes after 800 s by addition of detergent. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

bilayer nor did it change when the acceptor $ColA_{166}$-647 was present in a 1:1 M ratio. Since we could not find any evidence for FRET under the applied conditions, we can conclude that ColA did not oligomerize after binding to the bilayer. After binding application of a trans-membrane potential of 100 mV resulted in channel openings of ColA. However, the donor lifetime remained constant, also under these conditions (Fig. 3D).

We also applied fluorescence cross correlation spectroscopy to determine whether $ColA_{166}$-488 and $ColA_{166}$-647 diffused as a bound complex. The diffusion times of $ColA_{166}$-488 and $ColA_{166}$-647 at the bilayer indicated constant binding of both binding partners (Fig. 3E). However, the cross-correlation amplitude was zero, implying that no complex formation between $ColA_{166}$-488 and $ColA_{166}$-647 occurred under the applied conditions.

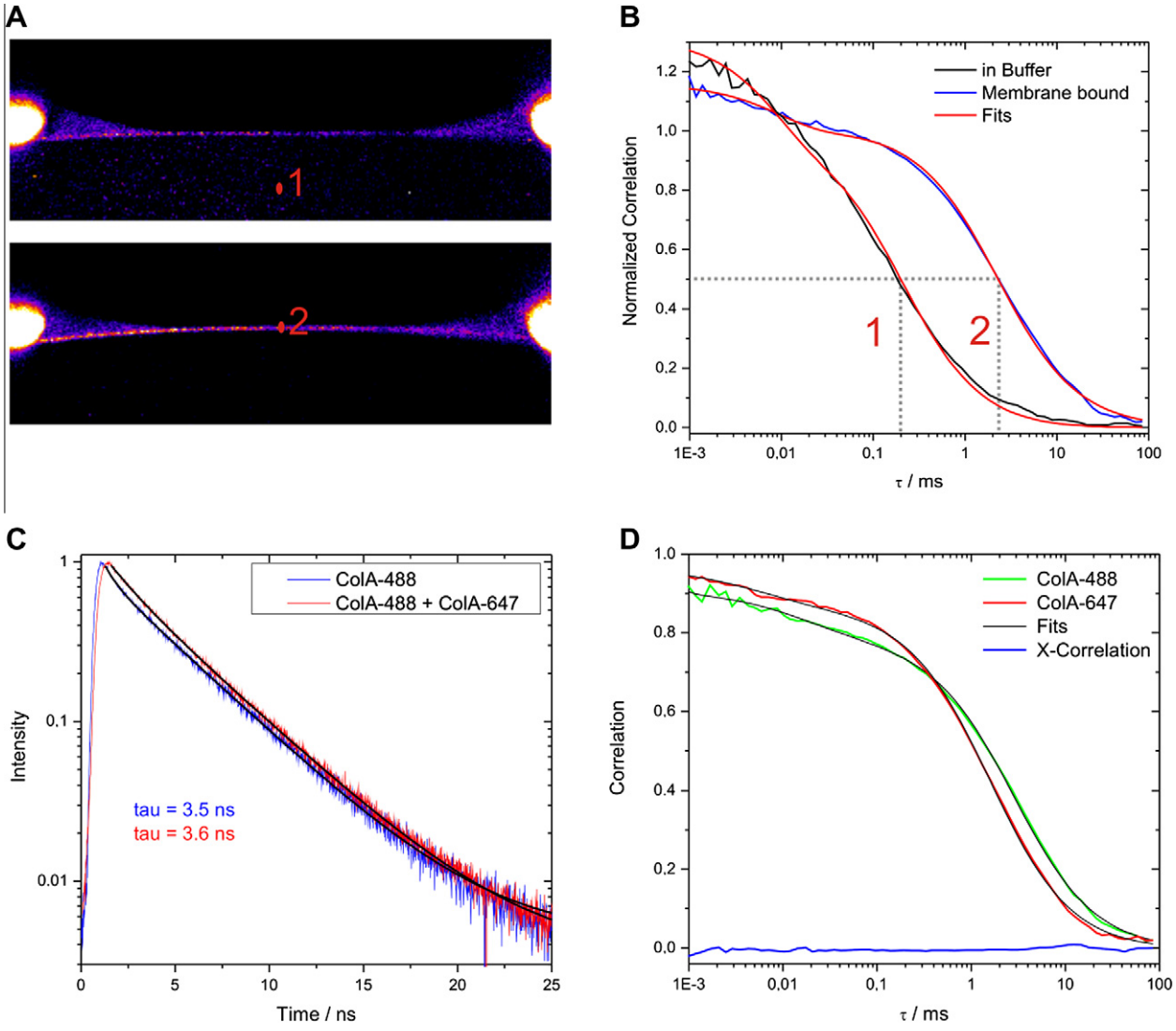

Fig. 3. Oligomerization measurements of ColA in HLBs. (A) Oligomerization measurements of ColA in HLBs (A) $ColA_{166}$-488 was added to trans in a concentration of <1 nM (position 1) to ensure a low surface density in the bilayer (position 2) after perfusion of trans. (B) FCS measurements at position 1 and 2 indicate a reduction of diffusive mobility of ColA166-488 upon membrane binding from 0.2 ms in solution to 2.5 ms at the bilayer. The fluorescence lifetime of the donor (ColA-488) bound to the bilayer was 3.6 ns. When the acceptor ($ColA_{166}$-634) was present in a 1:1 M ratio the lifetime of the donor did not change significantly, which indicates that no efficient FRET occurred under the applied conditions. (C) Cross-correlation between the donor (green) and acceptor (red) signals showed no correlation amplitude (blue trace) which indicates that the donor and acceptor molecules diffused independently from each other. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5. The membrane bound conformation of ColA

Membrane binding and integration of ColA was followed by the environment sensitive fluorescence probe NBD which was covalently attached to different cysteines introduced at specific sites of ColA (see Supplement Fig. 1A). Calibration of the NBD fluorescence lifetime dependence on the environment dielectric constant was achieved by determining the lifetime in six different dielectric environments (Supplement Fig. 1B). The lifetime of NBD increased exponentially with decreasing dielectric constants from 0.5 ns in water to 7.3 ns in n-decane.

Binding of ColA-NBD to the horizontal bilayer was determined by laser scanning the XZ-plane of the bilayer as depicted in Fig. 1B in the presence and absence of a membrane potential (ϕ_m = 100 mV). Channel opening was detected by monitoring the membrane conductance (Fig. 2C). After ColA membrane binding, all probed labeling sites (see Fig. S1A) were exposed to a more hydrophobic environment compared to the conformation in aqueous solution (Fig. 4A). Comparison with the lifetime calibration curve of NBD (Fig. S1B) reveals that all labeling sites were located at the membrane water interface. Mutant $E_{115}C$ which is located at helix 6 seemed to be most deeply immersed in the membrane. No significant differences between the lifetime, determined at zero potential and 10–20 min after application of 100 mV, were detected Fig. 4A).

4. Discussion

Despite the long history of colicin research the essential steps of the pore-formation process are still poorly understood. The main difficulty in obtaining structural information seems to be that the open channel conformation requires not only membrane binding of ColA but also a correctly oriented trans-membrane potential. In this study we used a combined electro-optical approach to characterize the membrane binding and channel-formation of ColA. We were able to determine the characteristic properties of the ColA pore forming domain such as pH dependent membrane binding

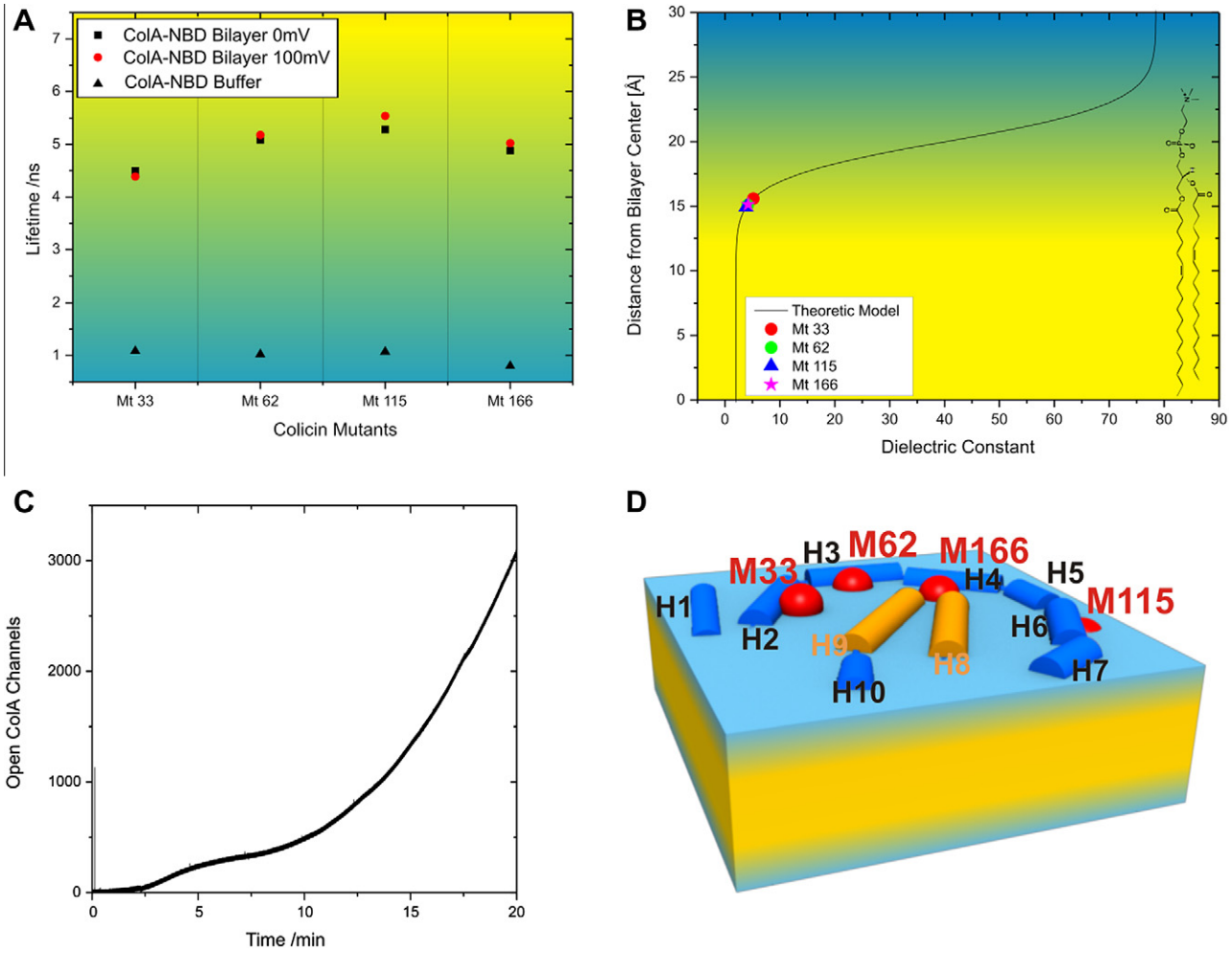

Fig. 4. Fluorescence lifetimes of ColA-NBD mutants. (A) Lifetime of ColA-NBD Mutants in aqueous buffer and bound to HLBs with and without a membrane potential (100 mV). (B) The bilayer immersion depth of ColA-NBD mutants was estimated from the lifetime dependence of NBD on the dielectric constant (see Fig. S1B) and a theoretical model of the dielectric map of the bilayer water interface [29,30]. All tested mutants resided at membrane water interface. (C) Number of open $ColA_{166}$-NBD open channels in the presence of a membrane potential (V_m = +100 mV). After 15 min ≈1000 ColA channels had opened (see (C)). (D) Membrane bound model of ColA estimated from the lifetime data.

and voltage dependent channel opening with a single measurement system (HLB-setup). Our results on the single channel properties and membrane binding agree well with what has been reported before [8,20–22]. While in the electrophysiological experiments the ColA channel appeared as a very low conductance narrow channel allowing only single file transport of small ions we observed in a transport assay that calcein was permeable revealing a ColA channel lumen with a diameter of at least 1 nm. The later observation is in line with previous observations using permeation assays of large anion/cations [9,23] as well as channel filling experiments with non-electrolytes of different sizes [24] and permeation of small folded proteins performed with other Col species [11]. Remarkably the calcein permeation through the ColA channel did not require application of a membrane potential.

4.1. Col A stays monomeric after membrane binding

We used three approaches to determine the oligomeric state of ColA after binding to the bilayer. Steady state FRET of liposome reconstituted ColA and lifetime FRET of ColA bound to horizontal lipid bilayers indicated that ColA remains monomeric after binding to the bilayer. Additionally, fluorescence cross-correlation spectroscopy of the FRET pair reconstituted in HLBs resulted in independent diffusion of the monomers. We can conclude that the majority (>98%) of the ColA population is monomeric after membrane binding at pH 4.

4.2. The membrane bound and open channel conformation of ColA

The fluorescence lifetime of NBD was used to probe the membrane immersion depth of four specific labeling sites in the pore-forming domain of ColA in the presence and absence of a membrane potential. Two conformations have been reported for the membrane bound ColA, the penknife conformation with all helices of the pore-forming domain located at the membrane water interface and the umbrella conformation with the hydrophobic helices 8 and 9 inserted in the bilayer. Our results show that, after membrane binding, all tested sites were exposed to an environment which was moderately hydrophobic implying that all tested regions were oriented at the membrane water interface.

Membrane immersion of all tested residues did not significantly change after application of a membrane potential. This could mean that none of the tested sites is involved in the conformational changes during channel opening. However, this possibility seems unlikely, because rather large scale conformational changes are required to produce an open channel from the membrane bound conformation. Formation of a channel with a diameter of ~1 nm by the monomeric ColA would require the membrane immersion

of at least 5 transmembrane helices as proposed earlier [28]. One possible reason why no changes in the membrane immersion depth of the NBD-probe were detected is that the in the membrane-bound and membrane-spanning state "have similar dielectrics and can therefore not be discriminated by lifetime measurements".

Acknowledgments

Excellent technical assistance from Birgit Hemmis is gratefully acknowledged. This work was financially supported by grants from the Deutsche Forschungsgemeinschaft (RW).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.09.069.

References

[1] F.G. van der Goot, Pore Forming Toxins, Springer Verlag, Berlin, Heidelberg, 2001.
[2] R.J.C. Gilbert, Pore-forming toxins, Cell. Mol. Life Sci. 59 (2002) 832–844.
[3] M.W. Parker, J.P. Postma, F. Pattus, A.D. Tucker, D. Tsernoglou, Refined structure of the pore-forming domain of colicin A at 2.4 A resolution, J. Mol. Biol. 224 (1992) 639–657.
[4] M. Wiener, D. Freymann, P. Ghosh, R.M. Stroud, Crystal structure of colicin Ia, Nature 385 (1997) 461–464.
[5] J.C. Lazzaroni, J.F. Dubuisson, A. Vianney, The Tol proteins of Escherichia coli and their involvement in the translocation of group A colicins, Biochimie 84 (2002) 391–397.
[6] E. Cascales, S.K. Buchanan, D. Duche, C. Kleanthous, R. Lloubes, K. Postle, M. Riley, S. Slatin, D. Cavard, Colicin biology, Microbiol. Mol. Biol. Rev. 71 (2007) 158–229.
[7] S.J. Schein, B.L. Kagan, A. Finkelstein, Colicin K acts by forming voltage-dependent channels in phospholipid bilayer membranes, Nature 276 (1978) 159–163.
[8] M.C. Martinez, C. Lazdunski, F. Pattus, Isolation, molecular and functional properties of the C-terminal domain of colicin A, EMBO J. 2 (1983) 1501–1507.
[9] L. Raymond, S.L. Slatin, A. Finkelstein, Channels formed by colicin E1 in planar lipid bilayers are large and exhibit pH-dependent ion selectivity, J. Membr. Biol. 84 (1985) 173–181.
[10] J.O. Bullock, E.R. Kolen, J.L. Shear, Ion selectivity of colicin E1: II. Permeability to organic cations, J. Membr. Biol. 128 (1992) 1–16.
[11] P.K. Kienker, K.S. Jakes, R.O. Blaustein, C. Miller, A. Finkelstein, Sizing the protein translocation pathway of colicin Ia channels, J. Gen. Physiol. 122 (2003) 161–176.
[12] S.L. Slatin, Colicin E1 in planar lipid bilayers, Int. J. Biochem. 20 (1988) 737–744.
[13] A.A. Peterson, W.A. Cramer, Voltage-dependent, monomeric channel activity of colicin E1 in artificial membrane vesicles, J. Membr. Biol. 99 (1987) 197–204.
[14] S.L. Greig, M. Radjainia, A.K. Mitra, Oligomeric structure of colicin ia channel in lipid bilayer membranes, J. Biol. Chem. 284 (2009) 16126–16134.
[15] P.V. Padmavathi, H.J. Steinhoff, Conformation of the closed channel state of colicin A in proteoliposomes: an umbrella model, J. Mol. Biol. 378 (2008) 204–214.
[16] A. Honigmann, C. Walter, F. Erdmann, C. Eggeling, R. Wagner, Characterization of horizontal lipid bilayers as a model system to study lipid phase separation, Biophys. J. 98 (2010) 2886–2894.
[17] A. Finkelstein, O.S. Andersen, The gramicidin A channel: a review of its permeability characteristics with special reference to the single-file aspect of transport, J. Membr. Biol. 59 (1981) 155–171.
[18] H. Felle, J.S. Porter, C.L. Slayman, H.R. Kaback, Quantitative measurements of membrane potential in Escherichia coli, Biochemistry 19 (1980) 3585–3590.
[19] S. May, Y. Kozlovsky, A. Ben-Shaul, M.M. Kozlov, Tilt modulus of a lipid monolayer, Eur. Phys. J. E Soft Matter 14 (2004) 299–308.
[20] V.L. Davidson, W.A. Cramer, L.J. Bishop, K.R. Brunden, Dependence of the activity of colicin E1 in artificial membrane vesicles on pH, membrane potential, and vesicle size, J. Biol. Chem. 259 (1984) 594–600.
[21] A.A. Musse, A.R. Merrill, The molecular basis for the pH-activation mechanism in the channel-forming bacterial colicin E1, J. Biol. Chem. 278 (2003) 24491–24499.
[22] V. Geli, M.C. Koorengevel, R.A. Demel, C. Lazdunski, J.A. Killian, Acidic interaction of the colicin A pore-forming domain with model membranes of Escherichia coli lipids results in a large perturbation of acyl chain order and stabilization of the bilayer, Biochemistry 31 (1992) 11089–11094.
[23] S.L. Slatin, A. Finkelstein, P.K. Kienker, Anomalous proton selectivity in a large channel: colicin A, Biochemistry 47 (2008) 1778–1788.
[24] O.V. Krasilnikov, J.B. Da Cruz, L.N. Yuldasheva, W.A. Varanda, R.A. Nogueira, A novel approach to study the geometry of the water lumen of ion channels: colicin Ia channels in planar lipid bilayers, J. Membr. Biol. 161 (1998) 83–92.
[28] R.M. Stroud, K. Reiling, M. Wiener, D. Freymann, Ion-channel-forming colicins, Curr. Opin. Struct. Biol. 8 (1998) 525–533.
[29] C.R. Sanders 2nd, J.P. Schwonek, An approximate model and empirical energy function for solute interactions with a water-phosphatidylcholine interface, Biophys. J. 65 (1993) 1207–1218.
[30] S. Mazeres, V. Schram, J.F. Tocanne, A. Lopez, 7-Nitrobenz-2-oxa-1,3-diazole-4-yl-labeled phospholipids in lipid membranes: differences in fluorescence behavior, Biophys. J. 71 (1996) 327–335.