Eur Biophys J (2000) 29: 153–157

BIOPHYSICS LETTER

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Interaction of phospholipid bilayers with polyamines of different length

Received: 19 November 1999 / Revised version: 25 February 2000 / Accepted: 25 February 2000

Abstract The tip-dip patch clamp method was used to study the effect of three polyamines, putrescine, spermidine and spermine, on bilayer lipid membrane (BLM) stability. Two kinds of mixed lipid-polyamine membranes were investigated. The poration voltage (V_p), and the closed (σ_{cl}) and open (σ_{op}) state conductances for pure and polyamine-treated lipid membranes were determined by the method of current-voltage surfaces. It was demonstrated that putrescine and spermidine destabilized lipid membranes under all circumstances. BLM stabilization by spermine was observed when it was added to preformed membranes.

Key words Path clamp · Putrescine · Spermidine · Spermine · Lipid-polyamine model membranes

Introduction

Polyamines are found in every cell of the body and have intricate mechanisms for release, uptake and metabolism. Despite many investigations of these compounds, their physiological function has remained an enigma for researchers.

The polyamines are simple aliphatic amines consisting of two or more flexible carbon chains that are connected by nitrogen atoms. The primary and secondary amine groups of polyamines always carry a charge at physiological pH, resulting in low molecular weight "organic cations". This polycationic quality led to the hypothesis that polyamines could affect living cells by binding to anionic sites, such as membrane phospholipids (Schuber 1989). The aim of the present investigation is to show the effect of varying the length of polyamines on the electric stability of model phospholipid bilayers.

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Materials and methods

L-α-Phosphatidylcholine from soybean, putrescine (Put) (1,4-butanediamine), spermidine (Spd) [N^1-(3-aminopropyl)-1,4-butanediamine] and spermine (Sp) [N^1, N^4-(3-aminopropyl)-1,4-butanediamine] were all obtained from Sigma and used as received. The structures of the polyamines are shown in Fig. 1. Hexane and KCl were Merck products. An aqueous solution of KCl (150 mM) was used for filling the patch pipettes and petri dishes.

Bilayer lipid membranes (BLMs) were self-assembled by the tip-dip method using patch pipettes and lipid monolayers from soybean lecithin (SL) (e.g., Petrov et al. 1991). Monolayers were spread from n-hexane solutions of SL (10 mg/ml). The seal resistance of the BLMs was controlled and only those with $R_{seal} > 1$ GΩ were used further. Two kinds of mixed lipid-polyamine membranes were investigated:

1. First kind: after SL BLM formation, water solutions of polyamines were added to the petri dishes (or to the surface of the SL monolayer) and then stirred for 5–10 min until there was a saturation of the transmembrane current. In this way we made sure that the polyamines were equilibrated with the previously formed SL BLMs.
2. Second kind: the BLMs were formed from premixed (SL + polyamines) monolayers with new patch pipettes.

Three different concentrations (10^{-2}, 10^{-3} and 10^{-4} M) of all the polyamine admixtures and one still lower (10^{-5} M) for putrescine (because of its high effect on lipid membranes) were investigated. The measurements were performed at room temperature (about 20 °C).

Conductance effects were recorded by a patch clamp amplifier using a computer-controlled voltage sweep DAC and a current-voltage surfaces' software (after Sansom and Mellor 1990). The principle of the current-voltage surfaces' method consists of simultaneous recording of the transmembrane current in response to a

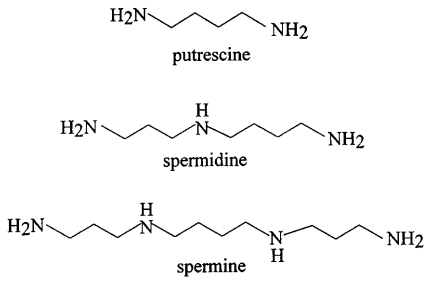

Fig. 1 Polyamine molecular structures

slow triangular ramp of the transmembrane voltage ($-V_m$ to V_m) for a time interval of typically 10 ramp periods, in bining I, V values in a 360 × 360 matrix in the I-V plane and in finally representing the number of events in each bin by 3–4 gray scale density levels. In this way, I-V curves belonging to closed or open conductance states were identified on the same compound I-V plot. Ramp amplitudes can be varied from 0 to 500 mV by steps of 50 mV.

Pure membranes were tested with voltage ramps from −10 to 10 mV up to −260 to 260 mV by steps of 50 mV. Voltages higher than 160 mV lead to a destabilization of

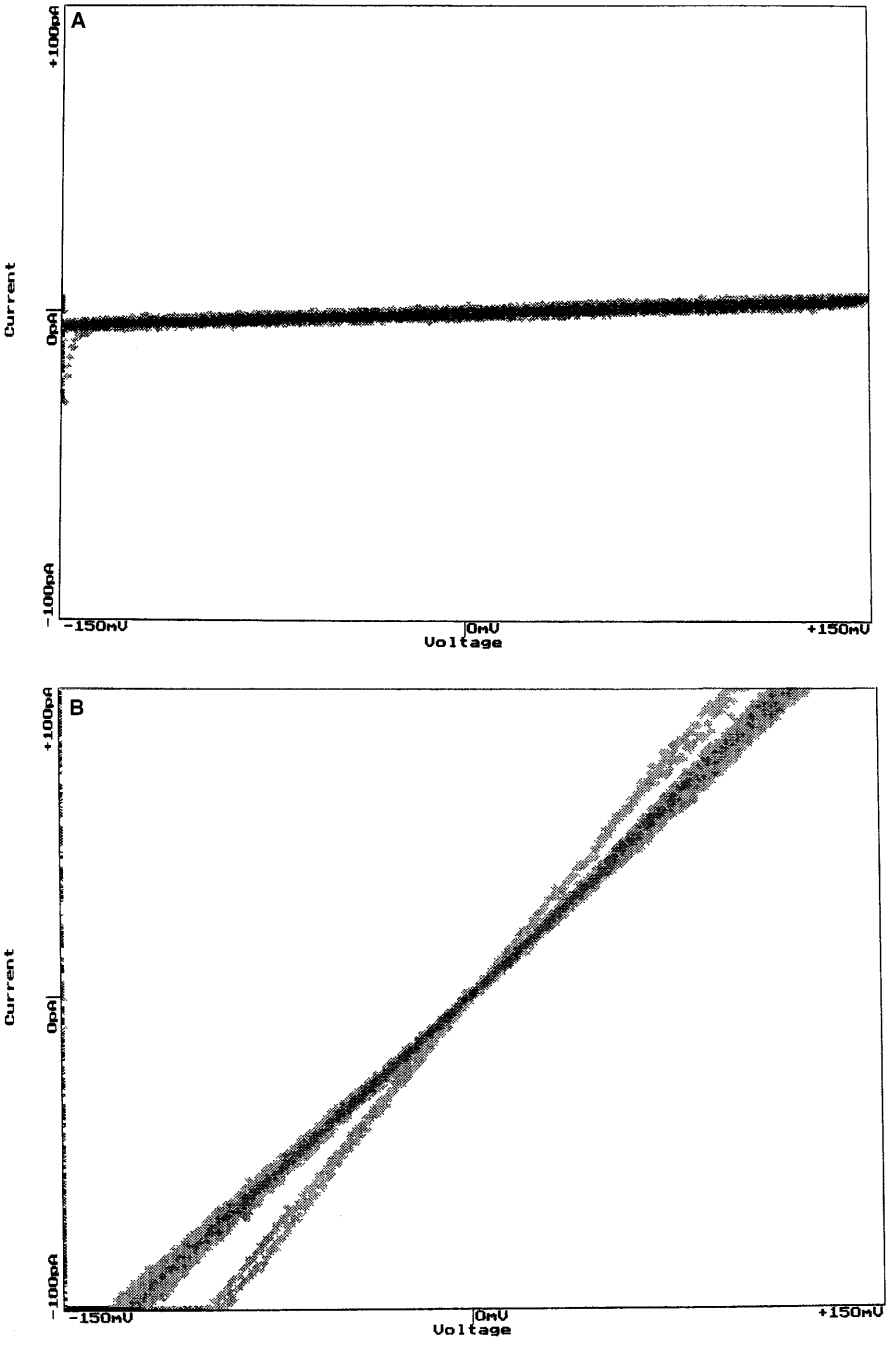

Fig. 2A, B Destabilization of a SL bilayer by putrescine. Current-voltage surfaces **A** of a pure SL bilayer and **B** of the same bilayer treated with Put. *Ordinate*, membrane current; *abscissa*, ramp voltage. Pipette resistance, 250 MΩ (150 mM KCl); 5 µl of 10 mg/ml SL/hexane spread over 5 ml KCl (150 mM); seal resistance 3 GΩ; −150 mV to +150 mV voltage ramp during 200 s. In **B**, three discrete pore conductance states emerge after adding an aqueous solution of Put to the bath to give final concentration of 10^{-3} M. Conductance states of 792, 1021 and 1098 pS were identified

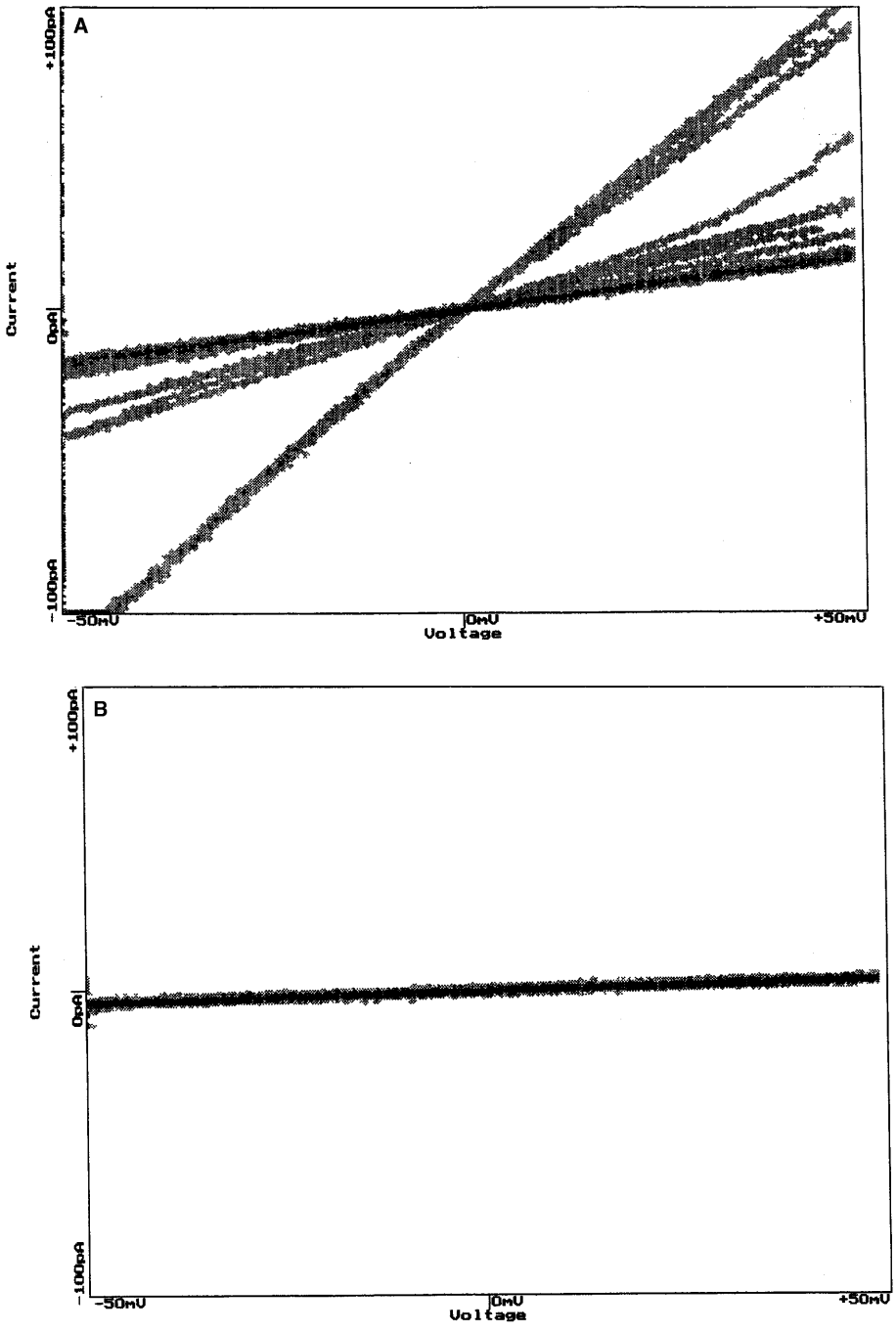

Fig. 3A, B Stabilization of a SL membrane by spermine. Current-voltage surfaces **A** of a pure SL bilayer and **B** of the same bilayer treated with Sp. *Ordinate*, membrane current; *abscissa*, ramp voltage. Pipette resistance, 250 MΩ (150 mM KCl); 5 µl of 10 mg/ml SL/hexane spread over 5 ml KCl (150 mM); −50 mV to +50 mV voltage ramp during 200 s. Several discrete conductance states are seen in **A**: 347, 490, 694, 698 and 2424 pS. In **B** the pore closed after adding an aqueous solution of Sp to the bath to give a final concentration of 10^{-4} M. V_p = 400 mV for the polyamine-treated membrane

pure lecithin membranes and the appearance of a pore or pores. Poration voltage is defined as the amplitude of that ramp when a pore, i.e. a higher conductance state, first appears in a particular membrane. The single pore conductance (σ_{op}) is defined as the slope of the *I-V* curve at that higher conductance state (presumably of a single pore, but the experiment gives no direct evidence), or as the tangent through the origin to the *I-V* curve, where it is nonlinear. After polyamine incorporation, mixed membranes were treated in the same manner, but now the destabilization appears at different voltages, either higher or lower (see below).

Results and discussion

An example of a current-voltage surface is shown in Figs. 2 and 3. Figure 2A represents a pure SL BLM. In this case the voltage was ramped between −150 mV and

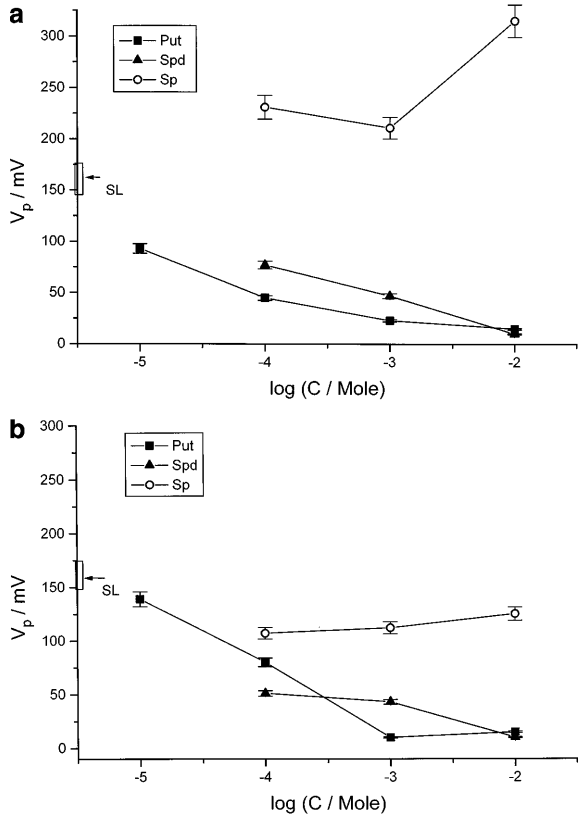

Fig. 4a, b Poration voltages versus concentration C (10^{-n} M) for the polyamine admixtures; n = 2, 3, 4 for Sp, Spd and n = 2, 3, 4, 5 for Put. **A** First kind of BLM. The comparison with the pure SL bilayer V_p (163 ± 16 mV) demonstrates a destabilization of the lipid membranes by Put and Spd and a significant stabilization by Sp. **B** Second kind of BLM. The comparison with the pure SL bilayer V_p (163 ± 16 mV) demonstrates a destabilization of the lipid membranes by all polyamines

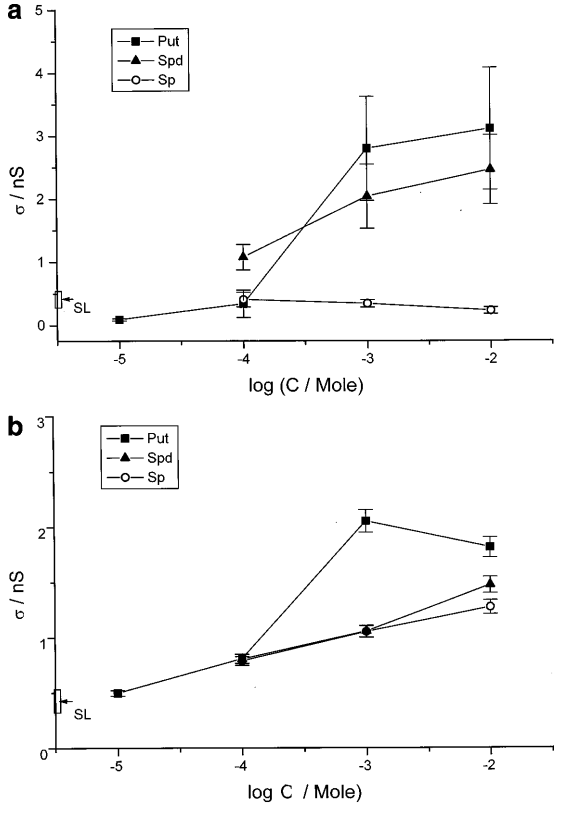

Fig. 5a, b Comparison of conductivities ($\sigma = \sigma_{op} - \sigma_{cl}$) versus the concentration C (10^{-n} M) for the polyamines admixtures; n = 2, 3, 4 for Sp, Spd and n = 2, 3, 4, 5 for Put. **A** First kind of BLM. **B** Second kind of BLM. For control, the pure SL membrane σ = 430 ± 104 pS is given on the ordinate

+150 mV. The same membrane response with the same ramp voltage, but after the addition of Put, is shown in Fig. 2B. Three conductance states are seen. Figure 3A represents a porated state of a SL membrane, as compared to a nonporated, stabilized state of the same membrane in Fig. 3B after the addition of Sp.

By stepwise changes of ramp amplitude the poration voltage (V_p) of all investigated membranes was determined with an accuracy of 50 mV. The closed (σ_{cl}) and open (σ_{op}) state conductances and V_p for pure and polyamine-treated lipid membranes were averaged over more than 10 experiments. A total of more than 400 membranes were investigated. For control SL BLMs the average V_p and $\sigma_{op} - \sigma_{cl}$ were 163 ± 16 mV and 430 ± 104 pS, respectively.

Figure 4 shows the poration voltages versus the concentration of the polyamine admixtures. Figure 4A shows the first kind of BLMs. The comparison with pure SL membrane V_p demonstrated a destabilization of the lipid membranes by putresine and spermidine (lower and decreasing V_p for increasing concentrations) and a significant stabilization by spermine (see also Fig. 3). Figure 4B shows the second kind of BLMs. The comparison with pure SL membrane V_p demonstrated a destabilization of the lipid membranes by all polyamines used, including spermine (in agreement with Spassova et al. 1998).

Figure 5 shows the (open-closed) state conductances versus the concentration of the polyamines. For the first kind of BLMs (Fig. 5A) the conductance of (SL + Sp) bilayers is almost the same like those of pure SL membranes. In the case of the Put and Spd admixtures, $\sigma_{op} - \sigma_{cl}$ is higher at higher polyamine concentrations and it is almost the same as the $\sigma_{op} - \sigma_{cl}$ of SL membranes at concentrations below 10^{-3} M. For the second kind of BLMs (Fig. 5B) the conductance of all mixed membranes is higher than those of pure SL membranes.

The reported result clearly demonstrate that polyamines do interact with phospholipid membranes. The specific mode of interaction (stabilization versus destabilization) is length dependent: species containing two and three amine groups destabilize the membranes under all circumstances, while species with four amine groups result in stabilization when added to preformed membranes.

Acknowledgements The authors are greatly indebted to Prof. P.N.R. Usherwood and Dr. I.R. Mellor for very helpful discussions.

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