Biophysical Journal, Volume 97

**Supporting Material**

# INTERACTION OF HEPARINS AND DEXTRAN SULFATES WITH A MESOSCOPIC PROTEIN NANOPORE

Luciana R. Teixeira, Petr G. Merzlyak, Angela Valeva, and Oleg V. Krasilnikov

*Supplemental:*

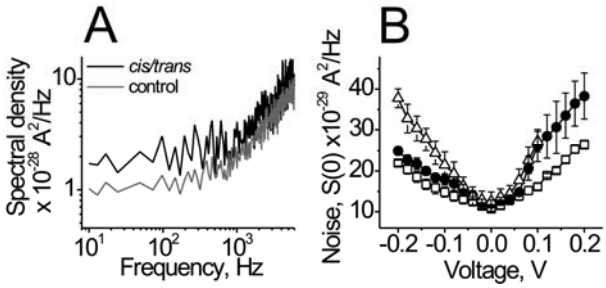


**Figure 1S.** Low-frequency power spectral density of the heparin-induced current fluctuations of the fully open single αHL channel.

**A.** The typical power spectral densities in the absence and in the presence of Hep3000 (400 μM *cis/trans*) at -100 mV applied voltage. It exceeds the control values by ~2 times.

**B.** Voltage dependencies of the spectral density averaged over 100 Hz – 1000 Hz range. Data are reported as means ±SD obtained in 5–7 independent experiments like those shown in **A**. Lipid bilayers of ~40 pF capacitance were used. Symbols: □ - control; ● - *trans*; Δ - *cis/trans*. The increase in the spectral density at high frequencies comes from the input amplifier and electrolyte noise.

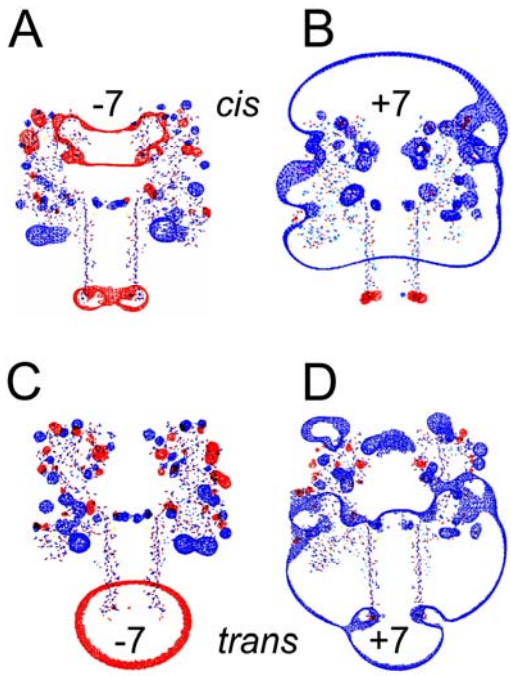


**Figure 2S.** The distribution of electrostatic potential at αHL channel formed by I7C (**A,B**) and T129C (**C,D**) after derivatizing the cysteines with DTNB (**A,C**) and MTSET (**B,D**). Cross-section through the αHL channel (Protein Data Bank, 7AHL.pdb) with electrostatic potential distribution shown in red (negative) and blue (positive). The potential was calculated using Coulomb calculation method (Swiss-PdbViewer version 3.7) assuming solvent ionic strength of 0.15 mol $dm^{-3}$.

**Table 1S. Activity of different polyanions against αHL channel.**

| Molecular type | Polyanion, kDa* | *cis*-side, IC50, μM | *trans*-side, IC50, μM |
|---|---|---|---|
| HepDi | 0.563 | ~15100 | |
| Hep3000 | 3 | 12.87 ± 2.26 | 4.5 ± 0.05 |
| Hep6000 | 6 | 1.017 ± 0.007 | 0.00318 ± 0.00103 |
| Hep | 18 | 0.018 ± 0.003 | 0.000255 ± 0.0000166 |
| HepAlb | 31# | 0.00017 ±0.0007 | 0.000014 ± 0.000002 |
| Hep + Alb | 18 | 0.14 ± 0.005 | |
| Hep + PEG35000 | 18 | 0.41 ± 0.03 | |
| | | | |
| DS5 | 5 | 44 ± 6 | |
| DS10 | 10 | 0.72 ± 0.15 | |
| DS500 | 500 | 0.0034 ± 0.0006 | |

HepAlb is heparin-albumin (H-0403) where, in accord with the supplier (Sigma), 4.8 mol heparin coupled through terminal formyl by reductive amination to 1 mol BSA;
Hep is heparin (as Na-salt) from porcine intestinal mucosa (H-9399), which has most chains in the range 17,000-19,000 Da with average molecular mass of 18000 g/mol;
Hep6000 is Heparin sodium salt from porcine intestinal mucosa (H-5284) with average molecular mass of 6000 g/mol;
Hep3000 is Heparin sodium salt from porcine intestinal mucosa (H-3400) with average molecular mass of 3000 g/mol;
HepDi is heparin disaccharides III-S sodium salt (H9392) with molecular mass of 563 g/mol;
DS500, DS10 and DS5 are dextran sulfates with average molecular mass of 500000 g/mol (D-6001); 10000 g/mol (D-6924), and 5000 g/mol (D-7037), respectively.

*- averaged molecular weight of polyanions; # estimated value.

*Relation to previous studies*

The present work clarifies how PA may affect mesoscopic ion channels. Previous studies with VDAC and αHL channel (3,4) found a nearly constant value for voltage-independent conformational energy between open and "closed" channel states at various PA concentrations and the great increase in effective gating charge. Based on conventional approaches, such effects were suggested to be a result of the direct (4) or indirect (3) electrostatic interaction between PA and the gating charges in the channels.

The results presented in the present study suggest that a single polyanion enters the pore due to electrostatic forces and physically blocks the ion conduction pathway, in contrast to mechanisms proposed earlier (e.g., via an increase in gating charges). Actually, the observed decrease in the multi channel membrane conductance or the probability to find a single channel in a high conductance state in the presence of PA is mainly result of plugging (what phenomenologically looks as a channel closing).

Obviously, that the probability of PA-ion channel interaction is dependent on PA concentration in the proximity of a channel entrances. It could be equal to or differ from that in the bulk solution. To estimate the value and explain the potential dependence of PA action, it was hypothesized (4) that depletion of anions at the negative potential end of the channel would develop a local positive potential and that PA partitions into this positive region where it can interact with a channel. In this case, value of the depletion, the local positive potential and PA partition itself is a transmembrane potential function. In this case, the build-up of PA there would be a dynamic and transient process, which starts with potential and dissipates soon after potential was switched to zero mV. The hypothesis has at least two weak points. First of all, even for highly conductive channel, like VDAC, to produce substantial ion depletion a channel should be surrounded with a relatively low electrolyte concentration and a high transmembrane potential should be applied for more than 10 s (1,2). Whereas, the authors (4) used high electrolyte concentration (1 M), only five mV and around a second to see full-effect of PA. Second, actually dynamic PA partitioning presented in the hypothesis must be slower with increase of a bulk solution viscosity. However, the experimental verification did not confirm that: the effectiveness even increased (3).
The current work suggests the non-linear Boltzmann law related polyanion distribution in the vicinity of a channel entrances seemingly as it was assumed by Mangan and Colombini (4). But unlike to those authors, which hypothesized that the distribution is determined by the transient (dependent on transmembrane potential applied) spot of positive potential on the front of a channel entrance, we assume the distribution is mainly 'static' and defined by force of a multi-pointed interaction of PA with membrane surface via $Ca^{2+}$ bridges, albeit obeys Boltzmann law too. Consequently, the greater the charge on PA, the larger should be it concentration near membrane. The importance of $Ca^{2+}$ bridges in polyanion effect on ion channel was suggested earlier (3), but the only protein part of a channel/membrane complex was assumed to be a target, while, as it shown in the present study, the polar heads of PC play the main role increasing the polyanion concentration close to membrane surface. In this case the polyanion distribution at a channel entrances does not depend on the transmembrane potential. It acts at the second step, where, a polyanion, occasionally approaching a channel entrance by diffusion, is either forced into or away from the pore by the transmembrane electric field. In addition, we showed that the charged residues of the protein moiety of the mesoscopic channel appear to modify the effective polyanion concentration in the proximity to the entrances interacting electrostatically with forthcoming PA.

Reference List

1. Chen, W. and R. C. Lee. 1994. Altered Ion-Channel Conductance and Ionic Selectivity Induced by Large Imposed Membrane-Potential Pulse. *Biophysical Journal* 67:603-612.
2. Cheng, L. J. and L. J. Guo. 2007. Rectified ion transport through concentration gradient in homogeneous silica nanochannels. *Nano Letters* 7:3165-3171.
3. Krasilnikov, O. V., P. G. Merzlyak, L. N. Yuldasheva, C. G. Rodrigues, and R. A. Nogueira. 1999. Heparin influence on alpha-staphylotoxin formed channel. *Biochimica et Biophysica Acta-Biomembranes* 1417:167-182.
4. Mangan, P. S. and M. Colombini. 1987. Ultrasteep Voltage Dependence in A Membrane Channel. *Proceedings of the National Academy of Sciences of the United States of America* 84:4896-4900.