Biophysics of nucleotides interactions with protein nanopores

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Abstract: - Physico-chemical condition of the electrolyte set inside the synthetic and protein nanopores establish different environment path for the passing salutes than that of they receive in bulk.. Due to the flexibility of protein nanopores, they present a different dynamic conformational changes compared with the rather rigid synthetic/polymeric nanopores when they face and external electric field. Various synthetic and protein nanopores have been used to study, control, sense and manipulate the conduction of different solutes by means of both experimental and theoretical approaches.
Here, for the first time we have used OmpF porin channels, a native channel forming protein in outer membrane of *E.coli,* to study the interaction and possible passage of nucleotides experimentally and theoretically. It was found that the channel's gating pattern, the conductance levels, substates sizes and distributions and also its voltage sensitivity differed in the presence of different nucleotides. Furthermore, titration and changing voltage sensitivity of the channel the pattern of the channel's gating changed, indicating the possible involvements of luminal charges in nucleotides movement.. The symmetrical/asymmetrical behavior of the channel was also studied when the nucleotides were introduced from cis side of the channel show unidirectional effect of the nucleotide on channel activity.

Key-Words: -biophysics, ion channels, pores, nucleotides

1 Introduction

Channel forming proteins are the major constituent of the membranes that control the traffic of different solutes, including , ions, nutrients and chemicals across the impermeable bilayer.
There are very limited structures of membrane proteins have been defined at atomic level, compared with cytoplasmic soluble proteins, filed at protein data banks. The availability of these structures defined by x-ray crystallography, and NMR, have provided the appropriate basis to conduct more realistic study on the structural origin of the channels behavior at atomic level. As a result of the fast and powerful computers it is possible to conduct theoretical analysis of the channels' structure, to complement the experimental results. The limitation in the experimental analysis in terms of their low sensitivity and slow sampling rate has made the application of theoretical parallel studies rather inevitable.
The function of membrane proteins has been studied throw biochemical, electrophysiological, and cellular approaches. However, the detailed molecular dynamics of molecules that form channel, receptor, pump, carrier and so on were defined when a molecular approach was implemented. Construction engineered proteins site-directed mutagenesis provided the chance of manipulation of certain site in the structure of the protein. The detailed voltage clamp analysis of the resulted structures at single molecule level at real time, shed further light on the way the protein behaves. Of the main potentials of this approach one can point to the possibility of working on voltage sensitivity , conductance, gating pattern, pH sensitivity, the effects of various ligands or peptides, and so on. Further, the technique's real time recording ability, makes it one of the unique techniques, capable of sensing the response of the single channel to the desired factor once it was presented.
The selectivity of the protein channels is defined based on the structure of its water filled lumen, and more specifically at its constriction site,. The arrangements of different charged, polar, apolar, hydrophobic, etc., of the amino acids at the site set both longitudinal and cross sectional electric fields that affect on the way the solutes move through [1]. As a result the channel might act as cation or anion

selective and in case of loose arrangement, weak cation or anion selective channel. Having the solute passed through the entrance of the channel, the first filtering gate, that permits solutes with certain size and charges, it is unique arrangement of charges at the constriction zone or so called eyelet area that controls the traffic of molecules [2].

The dynamic of the channel is controlled by its conformation in general and the arrangement of the amino acids side chains within the lumen of the channel as well as their position on the external and internal mouth of the channel. Considering the ion channels as transistors that have to be biased in certain condition to work, we can analyze the conduction of ions through the channel, at certain voltage, temperature, pH and so on. Whatever, that affects on the setting, and the well defined pattern of the channel gating and current can be identified at molecular level and very short time scale (micro seconds). In reality there are different path of ion conduction acting together during ion conduction. The analysis of the conduction as well as rate constant is considered based on the level of the potentials each ion is sensed along the channel [3] and is consistence with what presented in the Eyring transition state theory [4, 5]. This concept is considered as fundamental facts in the molecular dynamics simulation of the ion conduction process, where the summation of the conduction and vlosity at different part of the channel produces the panoramic view of the ion movements [6, 7] rather than a static energy profile. The other approach that has proven to be more promising is the Langevin model that deals with the time scales, dielectric constant, hydration friction, electrostatic interaction between ions and so on in a more efficient way, though it considers a rigid structure for the channel [8]. In a different approach, in Continuum Theory, applying Poasson-Nernst-Plank equations (PNP), the movement of ions through the channel is not studied singularly but as a continuous diffusion with a certain potential.[9, 10] In addition to what considered in different theoretical approaches, one must note that the affinity of certain sites for the moving ions plays a crucial role in the speed of the conduction [11] and to some extent in the selectivity of the channel.

There have been various attempts to study the flow rate as well as the filtering affects of different pores, both synthetic and natural molecules. The diffusion of the solutes in most channels is carried out in the direction of solutes' concentration gradient by means of chemical potential. [12]. Some channels are specified for certain jobs, TSX to transport nucleosides [13], LamB for maltose and malodextrins through outer membrane of gram negative bacterial, VDAC [14, 15], for anions through mitochondria membranes and so on. Specific channels posses certain conducting path for the molecules they are specialized to pass, and their diffusion rate is well consistent with what considered in Michaelis-Menten mechanism [12,16,13] Alpha Hemolysine, a pore forming toxin that is made be bacteria with a molecular weight of has been widely used to study the passage of heavy metals as a stochastic sensor [17] and homo-polymer nucleotide acids [18]. It has been tried to identify the interaction and passage of oligonucleotides containing up to 18 nucleotides. VDAC channels has also used to identify the passage of the nucleotides and their interaction with the channel. Here, OmpF porin channel, a homo-trimeric membrane water filed channel forming protein was used as a nono pore to conduct the interaction and passage studies. The atomic structure of the channel has been defined by x-ray crystallography with a resolution of -- A [19,20,21]. The channel consists of three homo-monomers with a total molecular weight of about 120KDa. The barrel structure of each monomer is formed by 16 anti-parallel beta sheets, connecting to each other by means of 8 extracluular loops and 8 cytoplasmic turns [22,23,24]. The monomers with a height of about 60 Angstrom takes an area of about 27*38 Angstrom in the lane of the membrane It is week cation selective and arrangement of amino acids at the constriction zone with a preserved sequence of PEFGG amino acids forms a certain motif. The constriction zone of the channel that s formed by the folding of L3 loop into the channel lumen, leaving an opening of 7*11 Angstrom, discriminates the passing molecules by means of their

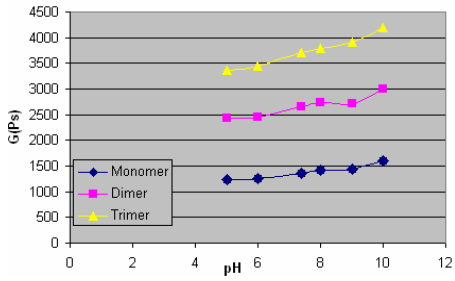
charges as well as size filtering, letting molecules with a molecular weight of less than 600 Da only [25]. The recent studies carried out by means of Atomic Force Microscopy [26] has further elaborated the structure function relationship in the channel at molecular level. The channel is voltage sensitive and conducts ions in an Ohmic manner and the relationship between the applied voltage and the caused current is linear up to voltage at which channel closes. The current carriers are potassium and chloride ions present in the medium buffer.
Nanopores were initially used by Coulter to detect blood cells in saline solution. This technique was later on further developed and using Resistive Pulse Technique, it was used to disnguish blood cells abnormalities based on their surface changes [27].

2 OmpF porin channel interaction with the applied nucleotides

Different effects of dTTP, dCTP, dGTP and dATP on the channel gating, conductance, voltage sensitivity and so on was studied by voltage clamp technique in planar artificial bilayes. Sampling intervals was 500 microsecond, signals were filterd by low pass filter up to 10 KHz and amplified with a gain of 100- 500 based on the circumstances. The current signals wee digitized and stored by computer and analyzed by Pat software.

2.1 OmpF porin channel activity

In order to study the channel activity first the OmpF porin channels were reconstituted into soybean artificial planar bilayer and their conductance, voltage sensitivity and gating pattern in general were studied.
Further to the analysis of the electrostatic interaction of the lining amino acids to the conduction solutes, the effect of pH on the channel activity was also studied. The results shows that the channels maximum conductivity takes place at alkaline pH, though at acid the channel tends to close and represent smaller conductance levels. The most stable condition of the channel was monitored at pH 5-9. At pH lower than 5, fast gatings with smaller amplitude in the resulting current peaks were recorded (Fig 1).



(Fig 1) OmpF conductance at different pH

The resulting conductance of monomer, dimmer and trimer of the channel has shown.

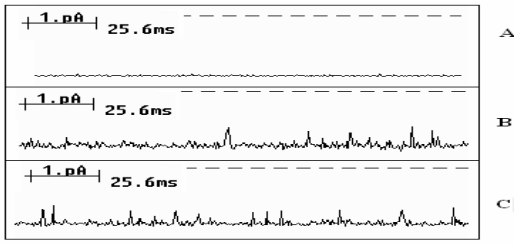
2.2 OmpF porin channel activity in the presence of nucleotides

The OmpF channel activity as a result of the presence of nucleotides at micomolar and milimolar concentration was studied following the addition of them into cis or trans side.

2.2.1 The effects of nucleotides on OmpF channel activity

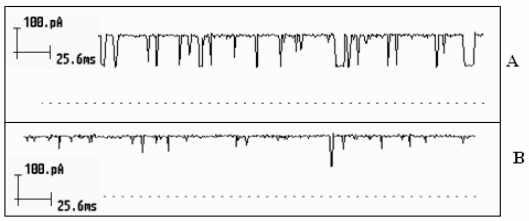
The instant effect of the added dTTP on the OmpF channel activity is shown in Figure 2.

As it is shown the channel gating pattern has changed dramatically due to the presence of dTTP mainly at negative polarity applied to the cis side, where the nucleotide was added.



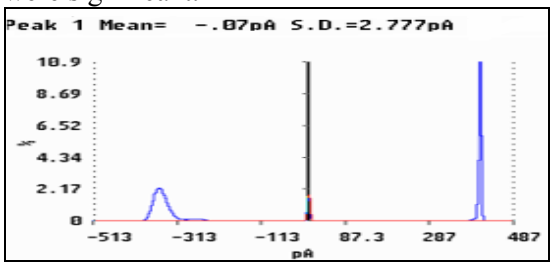
(Fig 2) The effect of dTTP (0.5 mM) on the gating of OmpF porin channel at different pH. Current at pH 7.4 (A), pH 7.4 and dTTP (B), and pH 10 and dTTP (C).

The channel gating pattern was also investigated as it is shown in Figure 3. The gating at negative and plosive polarities were analyzed by statistical parts of Pat software to further elaborate the differences.



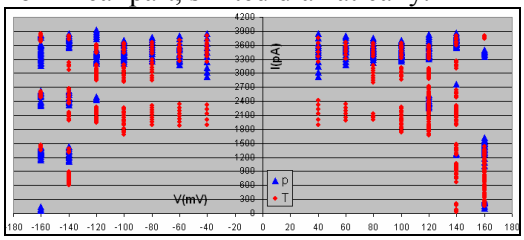
(Fig 3) OmpF channel activity in the presence of 2.1 μM dTTP in cis side of the channel at 120 mV (Top) and 140 mV(bottom).

Further, the conductance of the channel showed significant changes as appeared in Figure 4. The missing picks in and the distribution of the resulted conformation were significant.



(Fig 4) Different conductance levels of OmpF porin channels in presence and absence of dTTP.

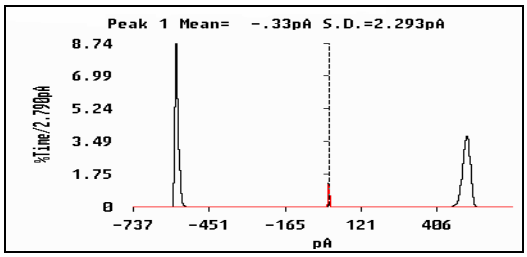
The current flowing through the channel at different membrane potential difference was also measured at different concentration of dTTP as it is shown in G/V curve in Figure 5. Although the linear relationship between the applied voltage and the resulting current still kept to some extent, i.e. the Ohmic behavior of the channel was still in place the non linear part, shifted dramatically.



(Fig 5) G/V curve of the OmpF porin channel in presence (P, in Blue) and absence (P, in red) of dTTP.

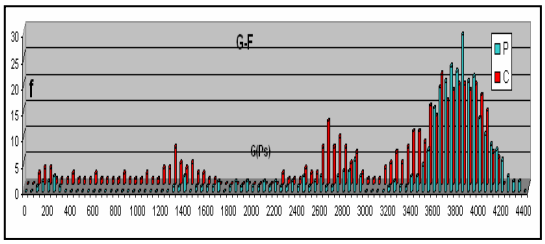
It is somehow an indication of the fact that the nucleotide changes the voltage sensitivity of the channel so that it gates at much higher voltages in the presence of dTTP.

The effects of other nucleotides were also studied. For example the effect of dCTP on channel gating is shown in Figure 6. The significant difference in channel gating at different polarity is repeated here too.



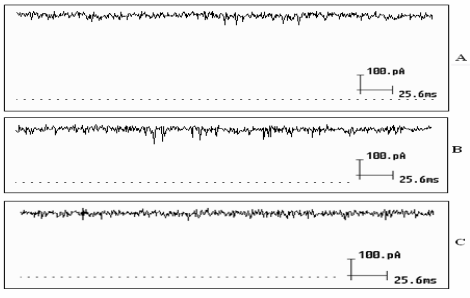
(Fig 6) The effects of dCTP on the conductance of OmpF porin channel at 100 mV.

The conductance levels produced by the channel as a result of the added dCTP is shown in Figure 7.

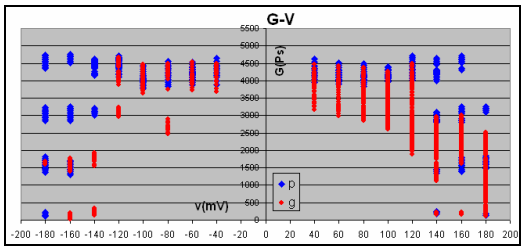


(Fig 7) Different conductance levels of OmpF porin channel in the presence and absence of dCTP. (bin=50, Np=306 ,Nt = 410)

The G/V curve of the channel activity (Fig 8) was produced based on the great deal of the data obtained from up to minutes recordings of different channel activities reconstituted in different membranes.



(Fig 8) OmpF channel current traces in the presence of dCTP.
The experiment was carried out analyzing the effects of dGTP (Fig 9, 10) .The effects of the added nucleotide on the conductance



(Fig 9) OmpF channel conductance levels in the presence and absence of dGTP

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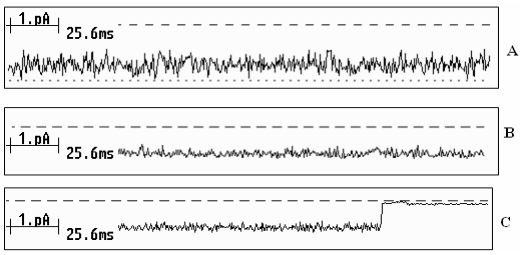
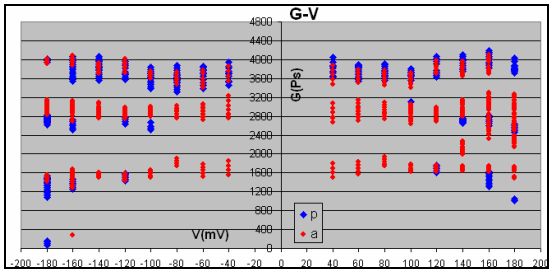
distribution, G/V curve and gating pattern of the channel at different polarity was quite difffeent from that of channel in the absence of dGTP.



(Fig 10) OmpF condaucance levels in the presence dGTP

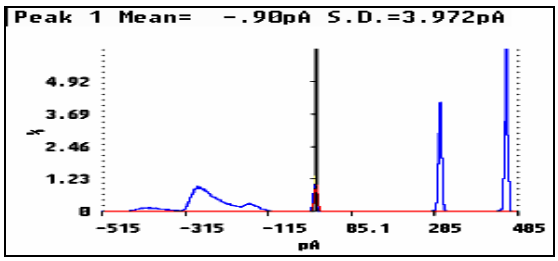
Analysis of dATP effects (Fig 11, 12) on the channel activities was also carried out by addition of micromolar and milimolar concentration of nucleotide into cis side of the chamber.

There was some similitarities in the channel behaviour identified between the G/V curve produced here with that of resulting as the addition of dTTP and dCTP. However, the shift in the conductance level distribution



(Fig 11) OmpF channel activity in the presence of dATP.

and formation of new states and substates was uniuque.



(Fig 12) OmpF conductance levels in the presence and absent of dATP.

In the last part of the experiment the effect of the pH on the OmpF channel activity was further studied and the resulted data were used to evaluate the electrostatic interactions between nucleotide and the channel's lumen amino acid side chains involved in the activities identified.

3 Discussion

Considering the sensitivity of the voltage clamp technique in analysis of single channel activity at real time, the analysis of nucleotide interaction with the channel and their possible flow through its lumen resulted in novel data presented here. Although, the elaboration of the electrostatic interaction at each specific site within the channel is promising (unpublished data) and is beyond the scope of the current article, the complementary article that present the theoretical analysis and statically summation of the data
Because of the effects of charges in the lining of the channel, it is possible to titrate some of them at certain pH, and facilitate the passage of nucleotides that might challenge moving through with more friction.
The G/V curves are well representing the involvement of the nucleotides with the voltage sensitive parts of the channel and also to some extent represents the energy required to overcome the negative effects of nucleotides on the channel's energy required to change its conformation, local or general.
The fact that the conductance of the channel produces different and even new conductance levels may present new arrangements and/or partial blockage due to the presence of nucleotides stable enough to be recorded.

4 Conclusion

Porin channel forming proteins whose 3D structures are known at atomic level are good candidate for biophysical molecular analysis. The clear idea of the way solutes pass interact with the channels is vital to conduct gene transferring, and transfection. Furthermore, to overcome the malfunction of the channels in the transportation of certain antibiotics, mainly taking place in MDR, Multi-Drug-Resistant bacteria needs to understand the channel solutes interaction at molecular level

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