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Q1 OmpF, a nucleotide-sensing nanoprobe, computational evaluation of single channel activities

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HIGHLIGHTS

- The real time gating activities of OmpF, is applicable for nucleotide recognition.
- The blockage occurred mainly with nucleotides and positive polarity set in cis side.
- Structure-Function and Permutation entropy distinguished nucleotides concentration.
- Variance/autocorrelation and Fractality distinguish some nucleotides from each other.

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ABSTRACT

The results of highthrouput practical single channel experiments should be formulated and validated by signal analysis approaches to increase the recognition precision of translocating molecules.

For this purpose, the activities of the single nano-pore forming protein, OmpF, in the presence of nucleotides were recorded in real time by the voltage clamp technique and used as a means for nucleotide recognition. The results were analyzed based on the permutation entropy of current Time Series (TS), fractality, autocorrelation, structure function, spectral density, and peak fraction to recognize each nucleotide based on its signature effect on the conductance, gating frequency and voltage sensitivity of channel at different concentrations and membrane potentials.

The amplitude and frequency of ion current fluctuation increased in the presence of Adenine more than Cytosine and Thymine in milli-molar (0.5 mM) concentrations. The variance of the current TS at various applied voltages showed a non-monotonic trend whose initial increasing slope in the presence of Thymine changed to a decreasing one in the second phase and was different from that of Adenine and Cytosine; e.g., by increasing the voltage from 40 to 140 mV in the 0.5 mM concentration of Adenine or Cytosine, the variance will decrease by one third while for the case of Thymine the variance will double. Moreover, based on the structure function of TS, the fractality of current TS differed as a function of varying membrane potentials (pd) and nucleotide concentrations.

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Accordingly, the calculated permutation entropy of the TS validated this biophysical approach for recognition of different nucleotides at various concentrations, pd's and polarities. Thus, the promising outcomes of the combined experimental and theoretical methodologies presented here can be implemented as a complementary means in pore-based nucleotide recognition profile.

1. Introduction

Sequencing of the patient's genome is essential to recognize the cause of disease(s), prescribe medicine and tailor precise and specific individual based clinical treatments. However, it is too expensive and takes too much time to be conducted by the current routine procedures. Thus there is a need to develop a high throughput screening technique that is lower in cost and more rapid. In our current study we have used a natural pore forming protein, OmpF, to conduct nucleotide recognition and the recorded results were formulated to pave the way for designing such a highthrouput technique. Several groups have conducted various studies to find efficient, fast and cheap approaches [1,2]. Accordingly, translocation of genetic materials through synthetic pores mounted on the electronic chips [3–13] as well as application of natural nanochannel forming proteins [14,15] have drawn much attention.

Most membrane proteins are involved in the translocation of various molecules including; amino acids and nucleotides [16,17], as well as molecules such as lactose [18]. Addressing the translocation of antibiotics through porin channels by theoretical and practical approaches [19–24] has made it possible to understand the mechanism(s) involved. Interaction of antibiotic with the OmpF channel have been experimentally evaluated and revealed the consequent closure of monomers [24]. Although, the translocation was shown to be sensitive to the polarity of the membrane potential, the time taken was invariant to the direction of the flow [25,26]. Ampicillin has been shown to have an affect on the average closure time of the monomer, changing it from 0.1 to 1.5 s in a temperature dependent manner (5–30 °C) [27].

Porins are channel forming molecules that due to their resistance in harsh experimental conditions are considered as potential candidates to be used as a means for sensing translocation in biosensors. They form hydrophilic channels that act as major gateways for translocation and uptake of different nutrients, molecules and ions across cell membranes. Their dynamic activities have been widely studied at the single molecule level in real time by experimental approaches [28,21,22,29,27,23,24,30,31] and provided the basis to understand and evaluate translocation aspects at the molecular level. Bacterial porins whose structures are known at the atomic level have attracted considerable attention [19,32,28,33–35,31] and were used to reveal the gating and translocation mechanisms. Molecular structures of many ion channels have also been studied by advanced theoretical and experimental methods [36]. OmpF is among the first ion channels whose structure was recognized by X-ray diffraction [37] and represented at atomic level [38]. The channel is a trimer, consisting of three homologous monomers (38KD) each formed from 16 anti-parallel beta strands that are connected to each other by means of 8 loops on the external side and 8 turns on the cytoplasmic side. One of the loops, L3, folds back into the channel and constricts it in the mid height of the channel, forming the so called "eyelet area" or "constriction zone" with an exclusion limit of 600D. The positive and negative charged amino acids located in the loop and neighboring barrel wall create a cross electric field that defines the selectivity of the channel for cation and anions, electrostatically.

Introduction of experimental single channel recording methods [39] has revealed dynamic activities of native membrane channel forming proteins and thus, showed their potential characteristics to be used as a real time nanosensor. Accordingly, we have used OmpF as a nanosensor to detect biological effects of the medium electromagnetic field on single protein molecules [40] and translocation of polynucleotides [41], and to elaborate on studies carried out by other research groups [6,42]. Kasianowicz and colleagues [14] have also investigated the translocation of polynucleotide RNA through a large α-hemolysin channel. It has been shown that recognition of solutes requires consideration and manipulation of many structural elements [43].

The translocation of molecules through channels has been also studied by theoretical and modeling approaches [44]. Different mechanisms are involved in the transportation process that includes induction of conformational changes caused through the binding process. Accordingly, structure-function relationships have been studied by both experimental [45] and simulation approaches [46]. Theoretical and fast molecular dynamic computational approaches (GROMACS, CHARM, NAMD, AMBER, LAMMPS) have provided a detailed insight about the structure, dynamics and functions of ion channels [47] at both atomic and molecular levels. The outcomes have provided reliable and applicable parameters [48–51,1,52] that are being used to further validate the results obtained from practical recognition approaches. Computational analysis has revealed various aspects involved in the gating events unique to each nucleotide at different time scales (Deng, X., Joseph, V.R. et al. 2009). In order to further validate the analysis, different statistical analysis, such as Permutation entropy has been used to measure the complexity in TS, distinguish periodic, random and chaotic TS [53,54] and Autocorrelation function [55] to reveal patterns hidden in the recorded signals. Furthermore, implementation of Structure Function of TS has revealed the Fractal structure of TS [56]. Fitting process, by means of the least square method with the Trust-region algorithm [57], has also been conducted to further analyze and reveal other parameters involved.

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