Links of conformational sampling to functional plasticity and clinical phenotypes by single molecule studies

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Published in:
Biophysical Society. Annual Meeting. Abstracts

DOI:
10.1016/j.bpj.2015.11.2144

Publication date:
2016

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Here, we describe discovery of two small molecules which specifically enhance catalytic efficiency of peptidase neurolysin. A computational approach was used to explore structure of neurolysin and identify a druggable surface pocket in its hinge region, followed by docking and ranking of 139,735 molecules from NCI DTP database. Top ranking compounds were subjected to pharmacological evaluation. Two structurally related compounds were identified which enhanced the rate of substrate hydrolysis by recombinant (human and rat) and mouse brain-purified neurolysin in a concentration-dependent manner. Neither the identified modulators nor dynorphin A(1-13), a competitive inhibitor, affected each other’s affinity in modulating activity of neurolysin, suggesting that the modulators do not bind to the substrate binding site. Both modulators reduced Km and increased Vmax values for hydrolysis of the synthetic substrate by neurolysin in a concentration-dependent manner. The modulators had negligible effect on catalytic activity of thimet oligopeptidase, nephrilysin, angiotensin converting enzyme (ACE) and ACE2, indicating that they are specific to neurolysin. Both modulators also enhanced hydrolysis of endogenous substrates, suggesting that their effect was not linked to the synthetic substrate. The identified molecules could be developed into research tools for evaluation of the (patho)physiological function of neurolysin. This study is one of few utilizing a structure-based approach for rational identification of enzyme activators, and by that it demonstrates applicability of this methodology for identification of allosteric modulators of other enzymes.

**1961-Pos Board B105**

**Links of Conformational Sampling to Functional Plasticity and Clinical Phenotypes by Single Molecule Studies**

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The dynamic exploration of conformational states, also termed conformational sampling, is known to govern all aspects of protein behavior from folding to function. The way however it encodes enzyme function - the capacity to accelerate the chemical step and plasticity of accepting structurally diverse substrates - are terra incognita for conventional bulk characterization but can be directly observed by single molecule studies (1-3). To interrogate the functional and structural dynamics of individual enzymes we spatially confine them on arrays of native membrane like systems (liposomes or nanodiscs)(4). In addition to providing a native-like environment - minimizing deleterious interactions with hard matter, these systems may act as 3D scaffold for the assembly of multiple regulatory bioelements. Our parallel single molecule readout allows us to directly observe, and quantify the extend of conformational sampling, is known to govern all aspects of protein behavior from folding to function. The way however it encodes enzyme function - the capacity to accelerate the chemical step and plasticity of accepting structurally diverse substrates - are terra incognita for conventional bulk characterization but can be directly observed by single molecule studies (1-3). To interrogate the functional and structural dynamics of individual enzymes we spatially confine them on arrays of native membrane like systems (liposomes or nanodiscs)(4). In addition to providing a native-like environment - minimizing deleterious interactions with hard matter, these systems may act as 3D scaffold for the assembly of multiple regulatory bioelements. Our parallel single molecule readout allows us to directly observe, and quantify the extend of conformational sampling acting as cue for functional and consequently clinical phenotypes.

**1964-Pos Board B108**

**Pressure Modulation of the Enzymatic Activity of Phospholipase A2 - a Putative Membrane-Associated Pressure Sensor**

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Phospholipases A2 (PLA2) catalyze the hydrolysis reaction of sn-2 fatty acids of membrane phospholipids and are involved in inflammatory responses to many neurodegenerative diseases through participation in receptor signaling and transcriptional pathways. We used pressure modulation of the PLA2 activity and of the membrane’s physical-chemical properties to reveal new mechanistic information on the membrane association and subsequent enzymatic reaction (1). Although the effect of high hydrostatic pressure on soluble and integral membrane proteins has been investigated to some extent, its effect on enzymatic reactions occurring at the water/lipid interface has not been explored yet. This study focuses on the effect of pressure on the structure, membrane binding and activity of membrane-associated PLA2. To this end, high pressure FTIR spectroscopy and high-pressure stopped-flow fluorescence spectroscopies were applied. The results show that PLA2 binding to model membranes is not significantly affected by pressure and occurs in at least two kinetically distinct steps. Followed by fast initial membrane association, structural reorganization of helical segments of PLA2 takes place at the lipid water interface. FRET-based activity measurements reveal that pressure has an inhibitory effect on the lipid hydrolysis rate. The reduced activity is due to a structural compression of the enzyme-substrate complex in comparison to that of the transition state. Additionally, a decrease in membrane fluidity upon compression impedes conformational changes accompanying various reaction steps, thereby reducing the rate of the overall reaction. Our results provide novel information regarding molecular interactions in the course of PLA2 binding and lipid hydrolysis, and are important for understanding how such enzymatic function is modulated by extrinsic factors such as those in the deep sea where pressures up to the kbar-level are encountered.