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BA.13 - Unveiling the mechanism of irreversible inhibition of aquaporin-10 by organogold compounds by biophysical methods and metadynamics

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Aquaporins (AQPs) are membrane protein channels that facilitate the diffusion of water and small solutes across cell membranes. For their involvement in a variety of pathologies, AQPs have emerged as potential drug targets, unveiling the use of selective AQP modulators as promising strategies for treatment of AQP-related diseases. Organometallic gold(III) complexes have gained interest with Auphen being discovered as a potent human AQP3 inhibitor. The mechanism behind this interaction was also reported, showing a reversible binding between the gold metal and AQP3 Cys40 residue. Here, we investigated the inhibitory effect of new organogold compounds in human AQP10 (hAQP10), an aquaglyceroporin expressed in the adipose tissue with relevance in body energy homeostasis. Using aqy-null yeast cells transformed with a plasmid encoding hAQP10, we tested the compounds' effect on glycerol permeability using stopped-flow fluorescence. Knowing that these compounds can react with cysteine residues and lead to the formation of stable and irreversible C-S bonds, we evaluated the reversibility of organogold compounds bond to hAQP10 cysteine residues, in the presence of β-mercaptoethanol. Moreover, we investigated their binding mechanism through molecular modelling and metadynamics atomistic simulations. Permeability assays revealed Au(III) CCON complex as one of the most potent of the cyclometalated Au(III) C^N compound series to inhibit hAQP10-mediated glycerol transport. These compounds revealed to irreversibly inhibit hAQP10-mediated glycerol permeability, probably due to the establishment of the stable covalent bond C-S. Computational results showed a local arylation of hAQP10 Cys209 residue by Au(III) CCON complex, resulting in alteration of the glycerol conductance pathway with overall shrinkage of the pore while water flux was barely affected. Thereby, even if the arylation occurs at a distance from the channel selectivity filters, the whole pore responds to this local modification. Altogether, we found Au(III) CCON complex as a potent inhibitor of hAQP10 glycerol permeability and identified a new mechanism of hAQP10 irreversible modulation by establishment of a covalent and stable C-S bond. **Keywords:** aquaporin, inhibitors, organogold compounds. **Supported by:** The authors acknowledge FCT - Fundação para a Ciência e Tecnologia, grant PTDC/BTM-SAL/28977/2017, fellowship 2020.04974.BD to C. Pimpão and projects UIDB/04138/2020 and UIDP/04138/2020 to iMed.ULisboa

BA.14 - Inactivation properties of ORIC, VRAC-like current of filamentous fungus *P. blakesleeanus*: the role of ATP and the first glimpse of the single channel behavior

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Outwardly rectifying, inactivating current (ORIC), recorded in cytoplasmatic droplets of *P.blakesleeanus* is characterized by a mild outward rectification, selectivity for anions, and a characteristic inactivation on depolarizing potentials. In absence of ATP, ORIC has a fast rundown with increasing speed of inactivation, while both processes are delayed by ATPpip (for at least 8min) and the non-hydrolysable AM-PCP (minimum of 5min). Extracellularly applied, ATP has a blocking effect. Flavonoid querzetin leads to a gradual loss of current despite the presence of intracellular ATP. Our research group has shown azide to be the potent respiration inhibitor of *P. blakesleeanus*, leaving the cells viable but significantly depleted from ATP. We are presenting data recorded in whole cell configuration, showing that: 1.ORIC can be activated despite the depletion of ATP in the cytoplasmatic droplet. However, its inactivation profile is significantly different comparing to normal conditions with or without ATP in the pipette in the first minute of recording, with reduced τ on lower depolarizing potentials. The fraction of current blocked by querzetin with AM-PCP in pipette solution is greater comparing to experiments with ATPpip , even with the 5-fold increase of AM-PCPpip concentration. The time required for current to recover from depolarizing steps is shorter at more hyperpolarized potentials. We have also started to look for the first insights into ORIC single channel activity in out-out configuration, and recorded, upon stimulation of ORIC in whole cell, and patch excision, the step-like pattern of inactivation that strongly resembles one of ORIC. Based on current data, we can conclude that ATP has a significant role in the inactivation process, with a more potent effect comparing to AM-PCP, whether by the means of greater affinity for the binding site or an additional mechanism involving kinase activity. **Keywords:** biophysics, ion, channels

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