7P.5 Membrane interaction of mitochondrial kinases: Mechanistic insights by analysis of thermodynamic and catalytic parameters

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xMitochondrial isoforms of creatine kinase (MtCK) and nucleoside diphosphate kinase (NDPK-D) have critical functions in bioenergetics, membrane topology and organelle morphology with roles in human health and disease [1, 2]. X-ray structural analysis, electron microscopy, surface plasmon resonance (SPR) and scanning calorimetry revealed that both kinases form large oligomers that bind to and cross-link mitochondrial membranes via anionic phospholipids, mainly cardiolipin [2]; at least MtCK can also induce cardiolipin-rich membrane domains. SPR with mitochondrial model membranes further showed a monophasic binding behavior of NDPK-D, depending on electrostatic interactions of a triad of basic amino acids [3], and biphasic binding of MtCK with predominant, but not exclusive, electrostatic interactions that were due to several C-terminal basic amino acids [4]. Here we performed a thermodynamic SPR analysis of the kinase/membrane interaction. Results showed (i) increased cardiolipin affinity of MtCK, but not of NDPK-D, with a rise in temperature, indicating participation of hydrophobic interactions; (ii) differing temperature-dependence of the rate constants characterizing the two binding phases of MtCK; and (iii) an entropy-driven binding process, possibly due to charge neutralization, release of bound water and effects on membrane order. Enzymatic activity of the fully membrane-bound kinases as compared to soluble controls showed no difference in case of MtCK, but strong inhibition in case of membrane-bound NDPK-D. This inhibition was relieved by treatment with doxorubicin that strongly competes for cardiolipin binding. Based on these data, we propose a model for MtCK and NDPK-D interaction with cardiolipin-containing lipid membranes. In case of NDPK-D, a single phase, purely electrostatic binding would lead to a partial shielding of the enzymes' active sites and thus catalytic inhibition. For MtCK, a two-phase binding model of rapid electrostatic docking and slower anchoring via hydrophobic stretches is proposed, which does not affect the active sites. This is consistent with earlier reports on additional hydrophobic interactions that reinforce the membrane-bound state of MtCK, but somehow disorder the lipid bilayer.

References

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7P.6 Isolation of putative respiratory strings or patches by blue-native electrophoresis on large pore gels

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Using our newly developed large pore acrylamide gels we could expand the application of blue electrophoresis to the separation of mega protein complexes with masses up to ~50 MDa. We identified 4–8 MDa assemblies that contain respiratory complexes I, III, and IV and most likely represent di-, tri-, and tetrameric forms of respiratory supercomplexes. We also isolated oligomeric respiratory supercomplexes in the mass range around 50 MDa, the presumed core pieces of respiratory strings or patches. The following observations support the presence of respiratory strings or patches: (i) oligomers are readily split into the individual complexes I, III, and IV under the relatively mild conditions of modified BNE — that makes random hydrophobic aggregation very unlikely; (ii) the identification of tetrameric complex IV but individual complexes I and III suggests a specific and ordered association of supercomplexes via tetrameric complex IV. Electron microscopic investigations will be required to verify the isolation of respiratory strings or patches.

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