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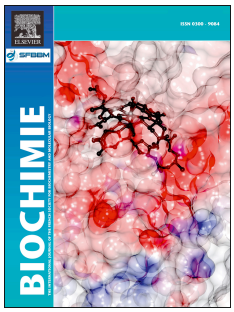

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Conservation of the oligomeric state of native VDAC1 in detergent micelles

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Abstract

The voltage-dependent anion-selective channel (VDAC) is an intrinsic β-barrel membrane protein located within the mitochondrial outer membrane where it serves as a pore, connecting the mitochondria to the cytosol. The high-resolution structures of both the human and murine VDACs have been resolved by X-ray diffraction and nuclear magnetic resonance spectroscopy (NMR) in 2008. However, the structural data are not completely in line with the findings that were obtained after decades of research on biochemical and functional analysis of VDAC. This discrepancy may be related to the fact that structural biology studies of membrane proteins reveal specific static conformations that may not necessarily represent the physiological state. For example, overexpression of membrane proteins in bacterial inclusion bodies or simply the extraction from the native lipid environment using harsh purification methods (*i.e.* chaotropic agents) can disturb the physiological conformations and the supramolecular assemblies. To address these potential issues, we have developed a method, allowing rapid one step purification of endogenous VDAC expressed in the native mitochondrial membrane without overexpression of recombinant protein or usage of harsh chaotropic extraction procedures. Using the *Saccharomyces cerevisiae* isoform 1 of VDAC as a model, this method yields efficient purification, preserving VDAC in a more physiological, native state following extraction from mitochondria. Single particle analysis using transmission electron microscopy (TEM) demonstrated conservation of oligomeric assembly after purification. Maintenance of the native state was evaluated using functional assessment that involves an ATP-binding assay by micro-scale thermophoresis (MST). Using this approach, we were able to determine for the first time the apparent K_D for ATP of 1.2 mM.