BIOCHEMICAL CHARACTERIZATION OF AN ATP-SYNTHASE FROM *POLYTOMELLA sp.*

Freire, M.M[1], Vázquez-Acevedo, M.[2], Scofano, H.M.[1], Mignaco, J.A.[1], Foguel, D.[1], Gonzalez-Halphen, D.[2]

[1] IBqM/CCS/UFRJ-RJ-Brazil
[2] IFC/UNAM-México

The mitochondrial FoF1-ATP-synthase from the non-photosynthetic alga *Polytomella sp.* presents novel subunits (ASA1-ASA9) structurally unrelated to other ATP-synthases. Some ASA compose the ATPase stator-stalk, while others stabilize the 1600 kDa dimer (Vázquez-Acevedo *et cols.*, 2006, J. Bioenerg. Biomembr. 38, 271-282). We are biochemically characterizing this *Polytomella* ATPase using permeable mitochondrial membranes. ATPase activity measured by hydrolysis of [γ-^{32}P]ATP (50mM Tris-Cl, pH 8.0, 2mM ATP, 2mM $MgCl_2$, 0.1mg.mL^{-1} mitochondrial protein, with/without 100mM KCl) was linear with time (1-60min), and proportional to mitochondria concentration. 0.01-0.1% Triton X-100 did not enhance activity, evidencing membrane permeability, but 0.5 and 1% Triton activated the enzyme by 50% and 30% respectively. KCl and alkaline pH (maximum at pH 8.5) activated the ATPase. Li^+/Na^+/K^+/Cs^+/NH_4^+/Chol-Cl (50-200mM) were equivalent activators. Mg^{2+} at 2mM (maximal rate) was preferred as divalent cation Mg^{2+}>Ca^{2+}>Zn^{2+}>Cu^{2+}>Fe^{2+}. At 5-10mM Mg^{2+} or Ca^{2+} were quasi-equivalent (90% maximal rate). 100µM DCCD or 100µg.ml^{-1} oligomycin (ca. 75-80%), 1mM vanadate (ca. 35%), and VO_4+DCCD (>90%) inhibited the ATPase. Resveratrol (100µM) was marginally inhibitory (<20%), while ouabain (1mM), thapsigargin (2µM) and bafilomycin (10µM) were ineffective. Preliminary parameters were V_{MAX}=1050 µmol.mg^{-1}.min^{-1}, K_M=0.90mM in presence and V_{MAX}=720 µmol.mg^{-1}.min^{-1}, K_M= 0.46mM in absence of KCl. Although it presents different subunit structure, *Polytomella* ATP-synthase bears conserved subunits and shares some distinctive functional features with orthodox FoF1-ATPases.

Sponsors: CNPq, FAPERJ (Brazil), CONACyT40696-Q, DGAPA-UNAM-IN218705-3 (México).

mailto: jmignaco@bioqmed.ufrj.br

Keywords: ATP-synthase, ATPase, mitochondria.