PRESSURE DISSOCIATION OF A DIMERIC ATP-SYNTHASE FROM *POLYTOMELLA sp.*

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The mitochondrial FoF1 from the non-photosynthetic alga *Polytomella sp.* is a dimer of oligomers with 1600kDa. Novel subunits (ASA1-ASA9), not identified in other ATP-synthases, participate of its second stalk and may contribute for dimerization. Incubation at 60°C for 20s dissociate the detergent-soluble enzyme to monomers; incubations >30s cause F_1 release and finally denaturation (Vázquez-Acevedo *et cols.*, 2006, J. Bioenerg. Biomembr. 38, 271-282). We now study the effects of hydrostatic pressure on the ATP-synthase. ATP-synthase was compressed on a quartz pressure cuvette, at 25°C. Conformational changes were followed by bis-ANS fluorescence, light scattering, ATPase activity, and BN-PAGE. The ATP-synthase did not dissociate below 1.4kbar. Above 1.4kbar a drastic conformational change (monomerization or dissociation of the holoenzyme) occurred, and ATPase activity decayed. Above 2.2kbar, although bis-ANS signal further increased, the enzyme was completely dissociated, because light scattering barely changed and ATPase activity was null. Upon decompression the enzyme reassociated imperfectly, because although the light scattering recovered, fluorescence and activity did not. BN-PAGE and HPLC confirmed that the protein reassociated to a dimeric mass, although some monomer-like and smaller complexes were observed. Dissimilar to pressure-dissociated ATP-synthase, heat-monomerized enzyme poorly bound bis-ANS, and did not reassociate upon cooling. The different pressure and heat-induced states of the ATP-synthase from *Polytomella sp.* might be useful to understand its subunit interactions.

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