Mammalian ATP synthase monomer vs dimer profiled by blue native PAGE and activity stain.

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A new and dynamic view of ATP synthase structure-function relationship is emerging, as the enzyme from mitochondria of different sources and from chloroplasts has been found in dimeric and higher oligomeric forms [1, 2]. The structural properties of these oligomeric forms are best characterized in yeast, where F₀ dimer-specific subunits (e, g) have been found to mediate monomer interactions along with subunit b [3, 4]. In bovine heart, monomers and dimers have identical subunit compositions [5], and the subunits involved in oligomerization are not known. Oligomerization is resulted independent of IF₁ binding to F₁ [6], as reported in yeast [7], but a recent study shows that in different mammalian cells, including bovine heart, IF₁ binding stabilizes the dimeric form most probably by bridging the two extramembranal F₁ parts [8].

Some clues to possible function and importance of dimers/oligomers are emerging from work carried out with the yeast enzyme, where it has been demonstrated that they play a critical role both in maintaining high transmembrane potential [9] and in determining cristae morphology [10]. In contrast, in mammals, the structural and functional roles of dimers/oligomers remain to be clarified.

A powerful approach for studying the ATP synthase supramolecular organization consists in one-step mild detergent extraction from biological membranes followed by blue native PAGE (BN-PAGE), which separates the different oligomeric states into abundant, clearly identifiable bands. Because the separated complexes remain active we optimized the in-gel ATPase assay to investigate the functional differences between monomers and dimers. We also used in-gel assay together with other electrophoretic and proteomic analyses to gain further insight into the relationship between oligomerization and activity, using mitoplasts (mitochondria denuded of outer membrane) and submitochondrial particles (MgATP-SMP).

In Triton X-100 extracts of heavy bovine heart mitochondria (HBHM) and mitoplasts, but not MgATP-SMP, dimers had greater specific activity than monomers: at 30°C, the dimer:monomer activity ratios were 2.3, 1.4 and 1.0, respectively. These differences in HBHM and mitoplast extracts were enhanced at 37°C but lost at 20°C. In mitoplasts but not in MgATP-SMP, dimers were selectively shielded from limited chymotrypsin degradation of F₁ α subunit, possibly due to interactions with other proteins or ligands in the native inner membrane. Despite these differences, all three preparations had similar percentages of dimers and similar contents of the native inhibitor IF₁ in Vm and Vd. These results suggest that, in native membrane, monomers and dimers are functionally distinct.