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PhD in Veterinary Sciences

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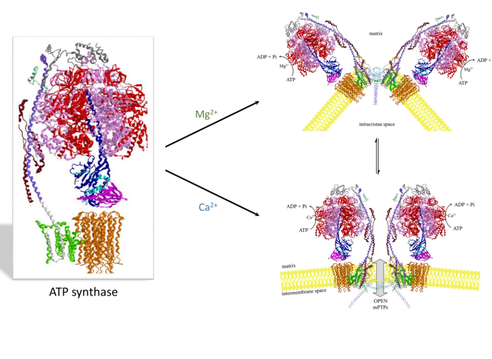
Modulation of the F_1F_O-ATPase and mitochondrial bioenergetics in species of veterinary interest

The F_1F_O-ATPase is an enzyme complex embedded in the inner mitochondrial membrane and composed of the hydrophobic (F_O) and the hydrophilic (F_1) domains, mutually connected by a lateral stator and an inner stalk. The enzyme can dimerize and oligomerize and the dimerization supports the curvature of the mitochondrial *cristae*. The enzyme is well known to catalyze the synthesis of ATP in aerobiosis, the high-energy molecule required by most life events. However, under certain physio-pathological conditions, accompanied by an increase in Ca^{2+} concentration in mitochondria, Ca^{2+} can bind to the enzyme and replace the natural cofactor Mg^{2+} [1]. Recent studies suggest that he Ca^{2+}-activated F-ATPase would trigger conformational changes which, once transmitted from F_1 to the membrane F_O domain, dissociate the dimer and open an high-conductivity channel, defined as mitochondrial permeability transition pore (mPTP) [2]. Since the mPTP opening initiates cascade events leading to cell death, most likely Ca^{2+} activates the lethal role of the enzyme.



The dual enzyme function, namely its vital role as main ATP maker and its lethal role as presumptive mPTP component, makes the F_1F_O complex an extremely attractive pharmacological target. Accordingly, mPTP modulators may be exploited to counteract mPTP-related pathological conditions such as neurodegenerative diseases, cancer, ischemia/reperfusion injury, muscular dystrophies [3] and mitochondrial dysfunction [4].

As first approach, on considering this intriguing perspective, this work aims at clarifying the molecular mechanisms by which Ca^{2+} binds to the F_1F_O-ATPase and the relationship between the Ca^{2+}-dependent hydrolytic activity of the enzyme and the mPTP opening. The study was carried out in heart mitochondria from pig, a widely exploited model in translational medicine.

◆ F-ATPase activation by Mg^{2+}, Mn^{2+} and Ca^{2+}

Fig.1

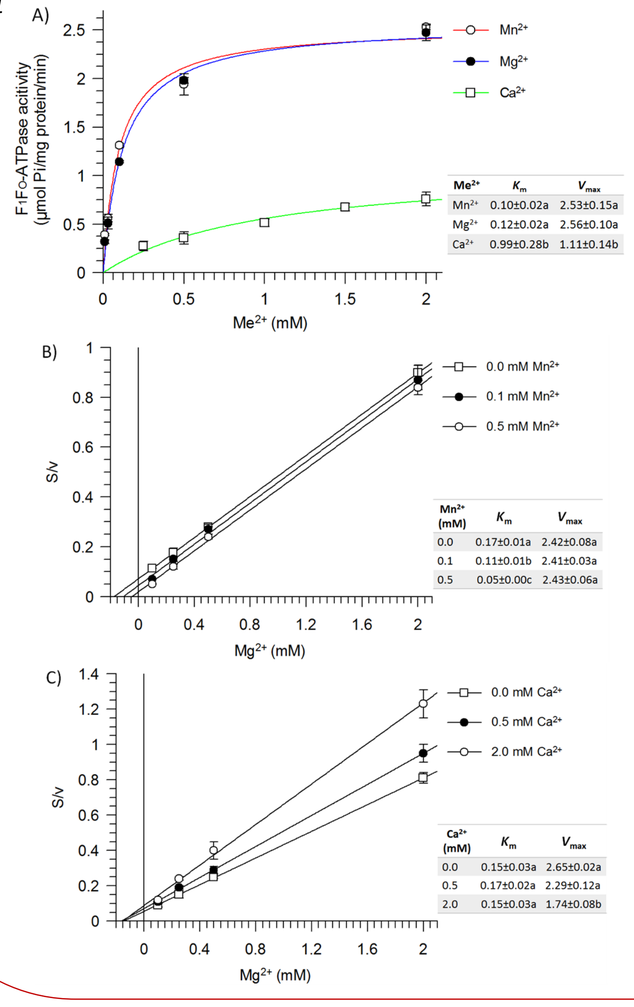


Fig.1 F_1F_O-ATPase activation by divalent cations. **A)** Michealis-Menten plot of Mn^{2+}, Mg^{2+} or Ca^{2+} dependent ATP hydrolysis. **B)** Hanes plots of the Mg^{2+}-F_1F_O-ATPase activity in the absence (□) or in the presence of 0.1 mM (●) or 0.5 mM (○) Mn^{2+} and **C)** in the absence (□) or in presence of 0.5 mM (●) or 2.0 mM (○) Ca^{2+}.
Data represent the mean ± SD from three independent experiments carried out on distinct mitochondrial preparations. Different letters indicate significantly different values ($P \leq 0.05$). The statistical analysis (ANOVA followed by SNK test).

◆ Inhibitors of the F_1 domain (Is) and inhibition parameters

Table 1

Is	ATP mM	Mg^{2+} mM	IC_{50}	k_i	k'_i	Inhibition mechanism
NBD-Cl	6	2	10.47 µM ± 0.35 a	-	4.58 ± 0.07 µM	uncompetitive
	6	0.5	10.90 µM ± 0.12 a			
	3	2	13.28 µM ± 0.56 b			
Piceatannol	6	2	119.61 µM ± 2.92 a	-	65.8 ± 1.2 µM	uncompetitive
	6	0.5	112.48 µM ± 5.82 b			
	3	2	187.10 µM ± 13.48 c			
Resveratrol	6	2	117.80 µM ± 8.74 a	-	56.6 ± 2.5 µM	uncompetitive
	6	0.5	101.32 µM ± 11.0 a			
	3	2	137.23 µM ± 16.86 b			
Quercetine	6	2	0.54 mM ± 0.03 a	-	0.64 ± 0.08 µM	uncompetitive
	6	0.5	0.60 mM ± 0.04 a			
	3	2	0.65 mM ± 0.07 a			

Table 2

Is	ATP mM	Ca^{2+} mM	IC_{50}	k_i	k'_i	Inhibition mechanism
NBD-Cl	3	2	3.82 µM ± 1.02 a	-	9.98 ± 0.95 µM	uncompetitive
	3	0.5	6.94 µM ± 0.68 b			
	1	2	9.06 µM ± 1.21 c			
Piceatannol	3	2	82.98 µM ± 4.67 a	19.5 ± 0.2 µM	76.6 ± 1.6 µM	Mixed competitve
	3	0.5	69.15 µM ± 19.72 a			
	1	2	63.07 µM ± 5.72 a			
Resveratrol	3	2	33.35 µM ± 2.12 a	-	27.5 ± 1.2 µM	uncompetitive
	3	0.5	38.07 µM ± 3.22 a			
	1	2	62.87 µM ± 2.52 b			
Quercetine	3	2	0.25 mM ± 0.03 a	-	0.30 ± 0.02 µM	uncompetitive
	3	0.5	0.25 mM ± 0.03 a			
	1	2	0.24 mM ± 0.02 a			

Table 1 shows the IC_{50} values for the F_1 inhibitors (Is) under different experimental conditions: optimal assay conditions (6 mM ATP , 2 mM Mg^{2+}), reduced cofactor concentration (6 mM ATP, 0.5 mM Mg^{2+}) and reduced ATP concentration (3 mM ATP , 2mM Mg^{2+}). K_i and K'_i values were obtained from the abscissa of the intercept of the straight lines in Dixon and Cornish-Bowden plots of the Mg^{2+}-activated F_1F_O-ATPase at 2 mM Mg^{2+} and 3 mM or 6 mM ATP.

Table 2 shows the IC_{50} values of the F_1 domain inhibitors under different assay conditions: optimal conditions (3 mM ATP, 2 mM Ca^{2+}), reduced Ca^{2+} (3 mM ATP - 0.5 mM Ca^{2+}) and reduced ATP concentration (1 mM ATP, 2mM Ca^{2+}). K_i and K'_i values were obtained from the abscissa of the intercept of the straight lines in Dixon and Cornish-Bowden plots of the Ca^{2+}-activated F_1F_O-ATPase at 2 mM Ca^{2+} and 1 mM or 3 mM ATP.

Each point represents the mean ± SD from three independent experiments carried out on distinct mitochondrial preparations. Different letters indicate significantly different values ($P \leq 0.05$). The statistical analysis (ANOVA followed by SNK test).

Fig.2

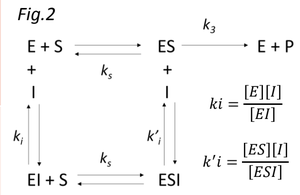


Fig.2 Representation of kinetic inhibition parameters

◆ Purification of the F_1 domain and response to inhibitors

Fig.3

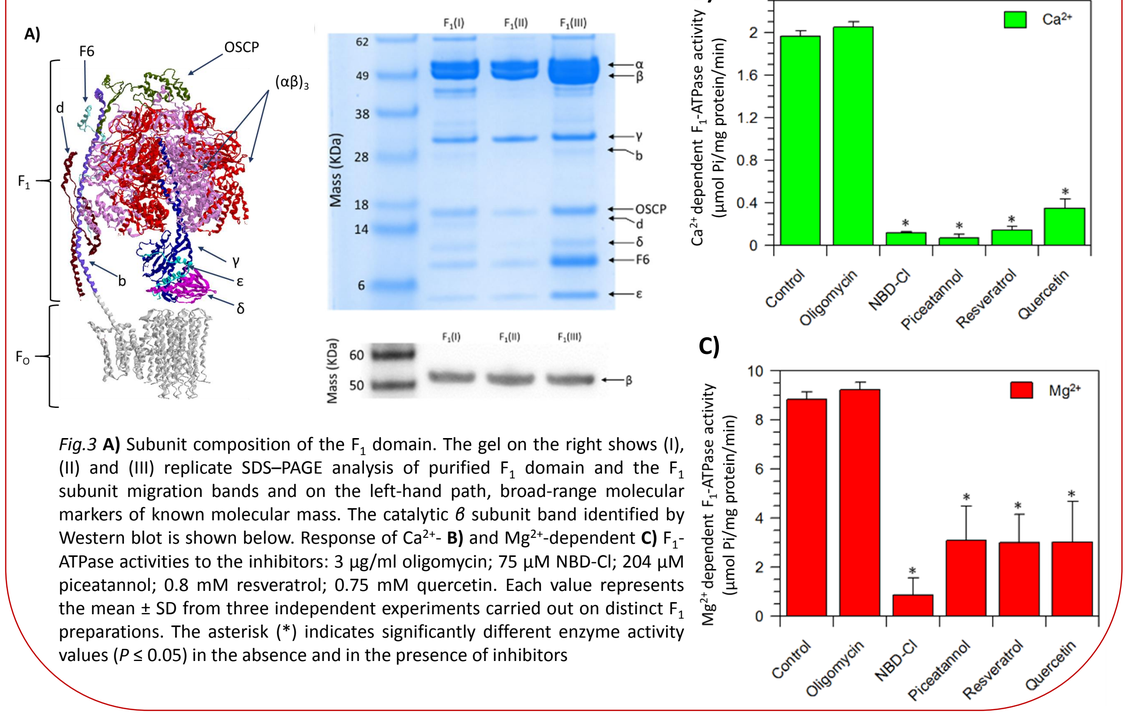


Fig.3 **A)** Subunit composition of the F_1 domain. The gel on the right shows (I), (II) and (III) replicate SDS–PAGE analysis of purified F_1 domain and the F_1 subunit migration bands and on the left-hand path, broad-range molecular markers of known molecular mass. The catalytic *β* subunit band identified by Western blot is shown below. Response of Ca^{2+}- **B)** and Mg^{2+}-dependent **C)** F_1-ATPase activities to the inhibitors: 3 µg/ml oligomycin; 75 µM NBD-Cl; 204 µM piceatannol; 0.8 mM resveratrol; 0.75 mM quercetin. Each value represents the mean ± SD from three independent experiments carried out on distinct F_1 preparations. The asterisk (*) indicates significantly different enzyme activity values ($P \leq 0.05$) in the absence and in the presence of inhibitors

◆ Evaluation of mPTP formation

Fig.4

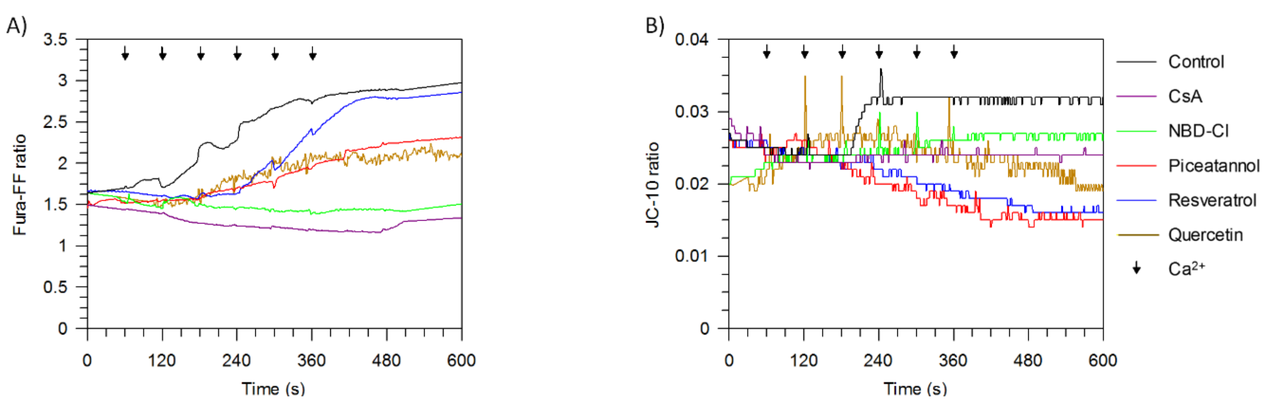
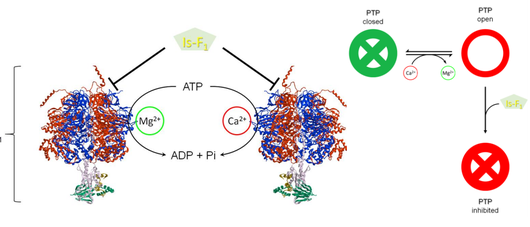


Fig.4 **A)** the mitochondrial calcium retention capacity (CRC) and **B)** the membrane potential (Δϕ) were detected in response to subsequent 20 µM $CaCl_2$ pulses, in the absence (Control) and in the presence of the inhibitors 1 µM CsA, 4 µM NBD-Cl, 80 µM piceatannol, 30 µM resveratrol, 200 µM quercetin.

Conclusion

The results confirm that Ca^{2+} binds to a bivalent cation site in F_1 and sustains ATP hydrolysis, even if by acting by a different mechanism from Mg^{2+}, probably due to the Ca^{2+} higher size than Mg^{2+}. Assumed that Ca^{2+} binding is responsible for the conformational changes in the enzyme complex which induce mPTP opening, the evidence that the inhibitors of the F_1 domain inhibit both the Ca^{2+}-dependent ATPase activity and the mPTP opening strongly sustain the hypothesis that the two mechanisms are tightly related.



References

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