Biophysical Journal, Volume 96

Supporting Material

Regulation of glycolytic oscillations by mitochondrial and plasma membrane H^+-ATPases

Lars Folke Olsen, Ann Zahle Andersen, Anita Lunding, Jens Christian Brasen and Allan K. Poulsen

Supplementary material to the paper

Regulation of glycolytic oscillations by mitochondrial and plasma membrane H^+-ATPases

Lars Folke Olsen[1], Ann Zahle Andersen[2],Anita Lunding[1], Jens Christian Brasen[1]*

and Allan K. Poulsen[1,3]

[1] CelCom and [2]Microbiology Group, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark, [3]Danish Technological Institute, Teknologiparken, Kongsvang Alle 29, DK-8000 Århus C, Denmark [*]correspondence: lfo@bmb.sdu.dk

Table S1 Effect of various inhibitors of glycolysis, respiration and H^+-ATPases on oscillations of NADH and mitochondrial membrane potential. (—) indicates inhibition; (+) indicates stimulation; (0) indicates no effect.

Inhibitor	Effect	Concentration added	Effect on NADH oscillations	Effect on $\Delta\psi$ oscillations
2-deoxyglucose	Inhibits hexokinase	5 mM	—	—
Iodoacetate	Inhibits glyceraldehyde-3-phosphate dehydrogenase	20 mM	—	—
Antimycin A	Inhibits complex III	2 μM	0	0
Azide	Inhibits complex IV Inhibits alternative respiration in S.l Cerevisiae Inhibits F_0F_1-ATPase	50 μM - 5 mM	—	—
SHAM	Inhibits alternative oxidases	4 mM	0	0
FCCP	Dissipates H^+ electrochemical gradients	50 nM-5 μM	0	—
Amphotericin B	Induces leakage of K^+ from cells	10-20 μM	—	—
Omeprazole	Inhibits plasma membrane H^+-ATPase	50-250 μM	0/?	+/—

Figure S1

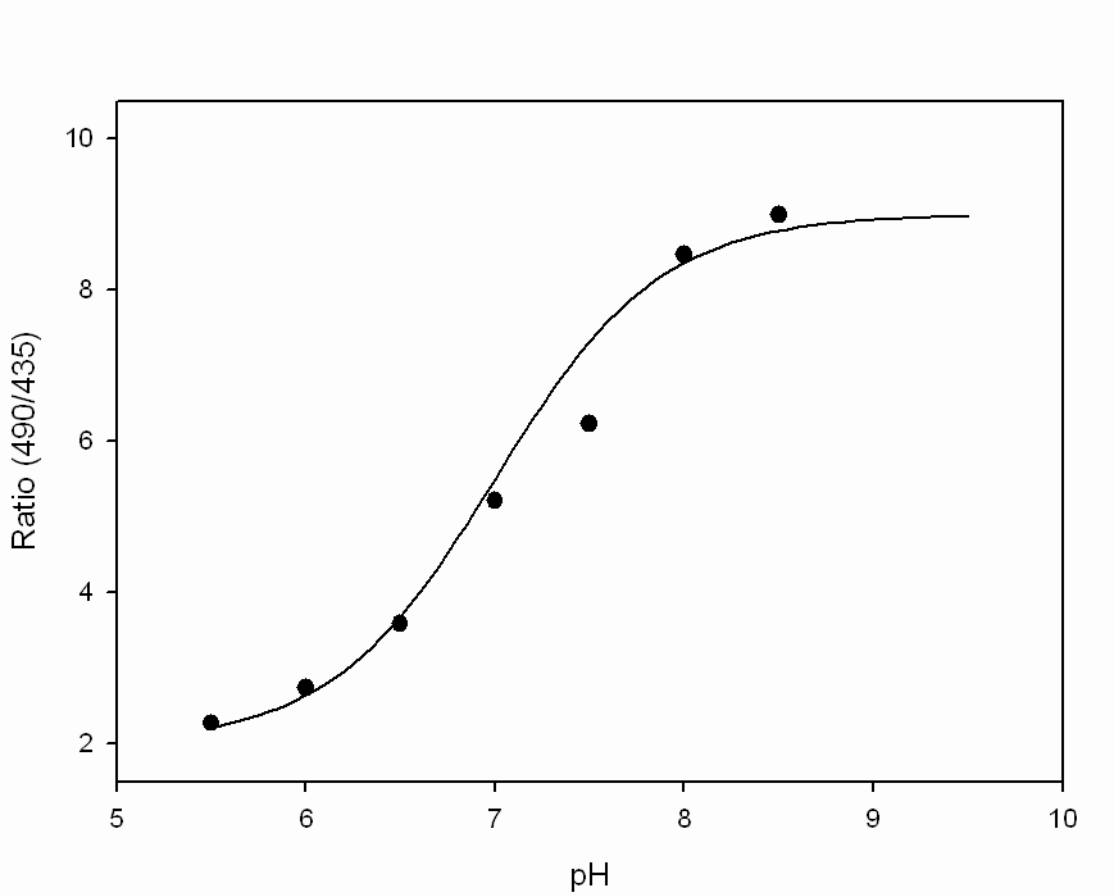

Figure S1. Calibration curve of fluorescence excitation ratio (490 nm/435 nm) vs. pH of permeabilized cells of *Saccharomyces cerevisiae* in citrate/phosphate buffer at different pH values. Permeabilization was done by suspending the cells in 70% (v/v) ethanol/phosphate buffer at 30 ^{o}C for 30 minutes. Then the cells were centrifuged at 7,000×g for 3 minutes and the pellet was resuspended in phosphate/citrate buffer at the desired pH. The permeabilized cells were stained with 5- (and 6-)- carboxyfluorescein diacetate succinimidyl ester at 40 ^{o}C for 20 min, centrifuged again, resuspended in the same phosphate/citrate buffer, and the excitation ratio (490 nm/435 nm, emission 520 nm) was recorded. Temperature 25 ^{o}C

Figure S2

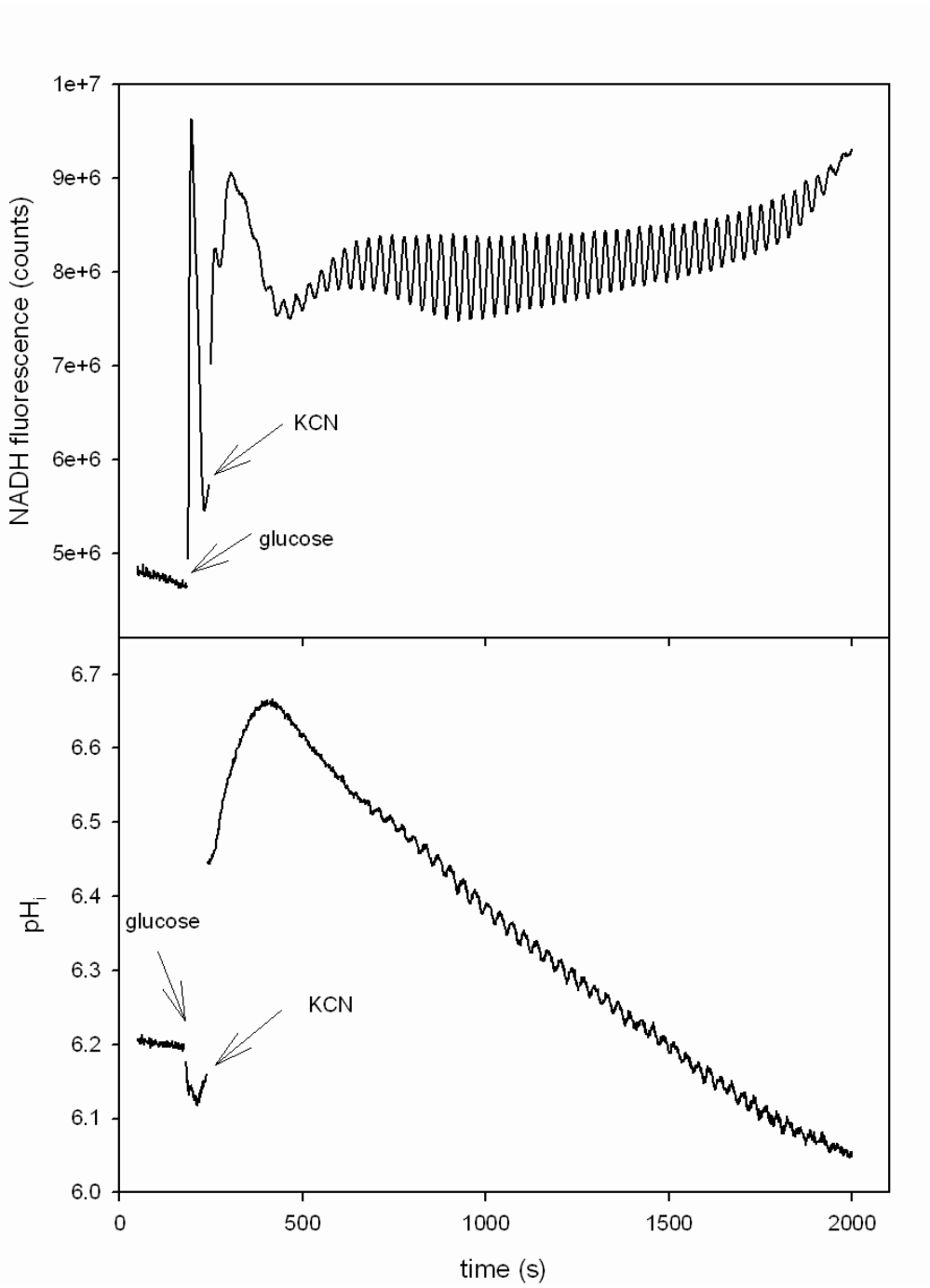

Figure S2 Time series of NADH fluorescence and intracellular pH in yeast cells stained with 5- (and 6-)- carboxyfluorescein diacetate succinimidyl ester and suspended to a cell density of 10% wet weight in 100 mM phosphate buffer at pH 5.5. At time t=180 s 30 mM glucose was added to the suspension followed by 5 mM KCN at 240 s; pH_i was determined as explained in the "Materials and Methods" section. The final external pH was 5.15 at 2000 s. Temperature 25 ^oC

Figure S3

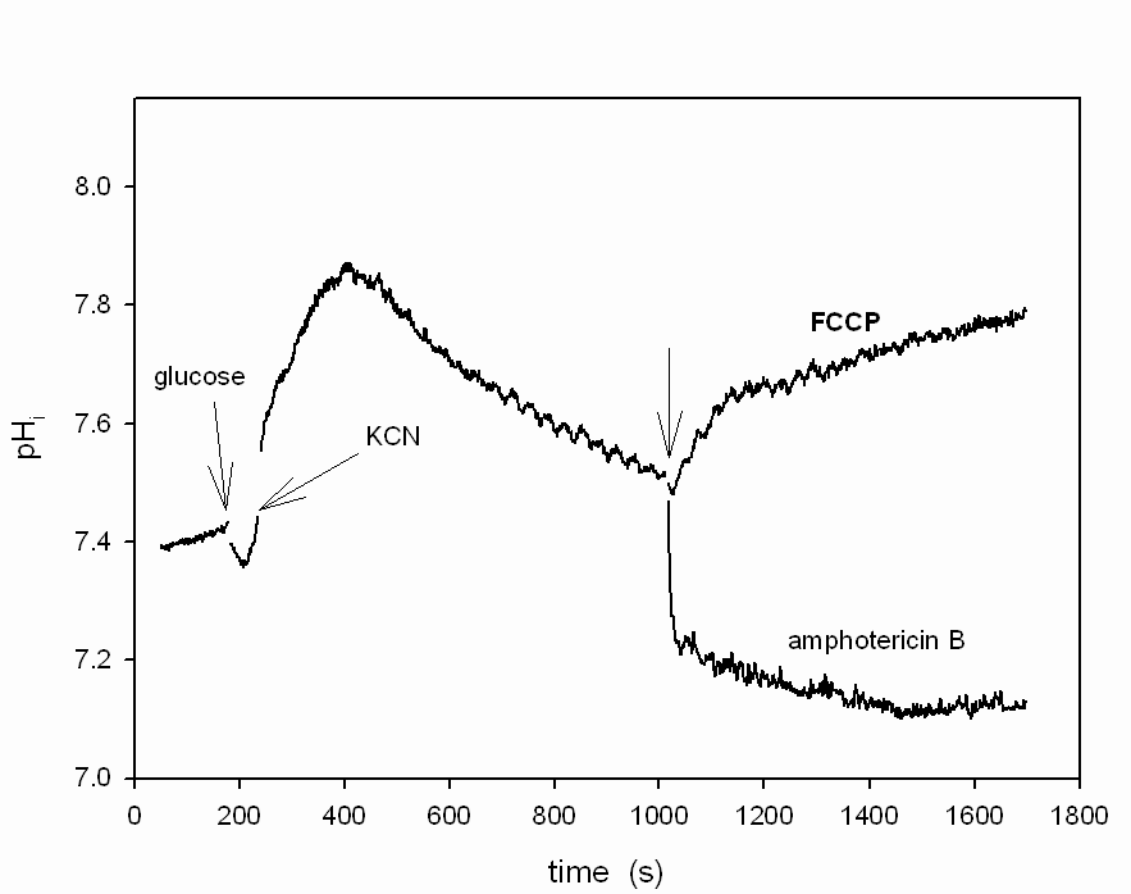

Figure S3. Effect of the addition of FCCP or amphotericin B on pH_i. Yeast cells stained with 5- (and 6-)- carboxyfluorescein diacetate succinimidyl ester were suspended to a cell density of 10% wet weight in 100 mM phosphate buffer, pH 6.8. At time t=180 s 30 mM glucose was added to the suspension followed by 5 mM KCN at 240 s. At time approximately 1000 s 20 µM FCCP or 20 µM amphotericin B were added to the suspension; pH_i was determined as explained in the "Materials and Methods" section. Temperature 25 ^{o}C

Figure S4

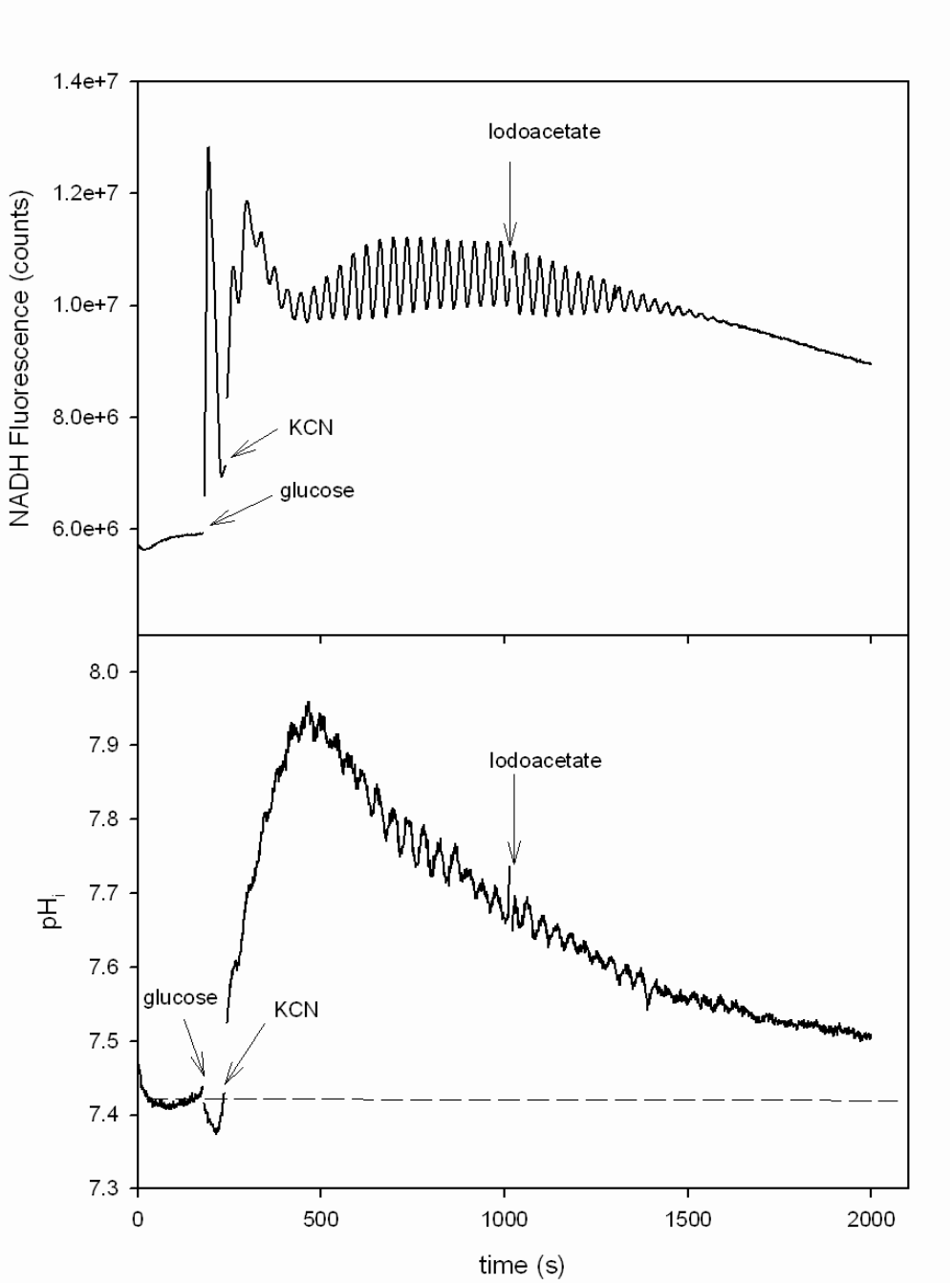

Figure S4. Effect of the addition of iodoacetate on pH_i. Yeast cells stained with 5- (and 6-)- carboxyfluorescein diacetate succinimidyl ester were suspended to a cell density of 10% wet weight in 100 mM phosphate buffer, pH 6.8. At time t=180 s 30 mM glucose was added to the suspension followed by 5 mM KCN at 240 s. At time approximately 1000 s 20 mM iodoacetate was added to the suspension; pH_i was determined as explained in the "Materials and Methods" section. Temperature 25 ^{o}C

Figure S5

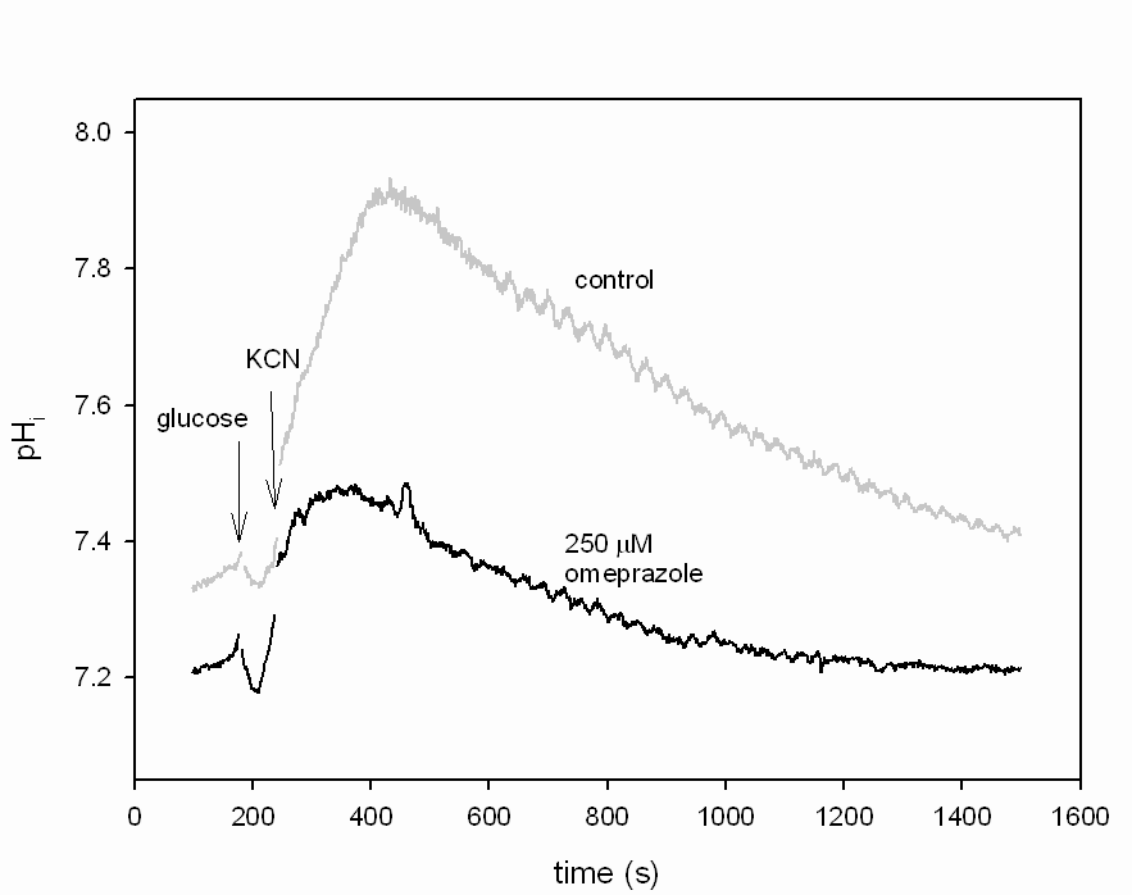

Figure S5. The effect of omeprazole on pH_i. Yeast cells (10% wet weight) suspended in 250 mM sorbitol were treated for 10 min with omeprazole as described in the "Materials and Methods" section. After centrifugation the cells were stained with 5- (and 6-)-carboxyfluorescein diacetate succinimidyl ester and suspended (10% wet weight) in 100 mM phosphate buffer, pH 6.8. The graphs show plots of the intracellular pH against time in the untreated (control) cells and cells treated with 250 μM omeprazole following addition of first 30 mM glucose and then 5 mM KCN as indicated. Temperature 25 ^{o}C