Detection of the Mitochondrial Potential Sensor JC-1
Tecan Ultra Evolution, Safire and GENios Pro

Introduction

JC-1 (Figure 1) is a cationic dye that exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (~ 525 nm) to red (~ 590 nm). Mitochondria depolarization is indicated by a decrease in the red to green fluorescence intensity ratio. The potential-sensitive color shift is due to concentration-dependent formation of red fluorescent J-aggregates. JC-1 can be used as an indicator of mitochondrial potential in a variety of cell types as well as in intact tissues and isolated mitochondria.

The ratio of green to red fluorescence is dependent only on the membrane potential and not on other factors such as mitochondrial size, shape and density that may influence single-component fluorescence signals. The fluorescence ratio detection allows comparative measurements of membrane potential to be made and the percentage of mitochondria within a population that respond to an applied stimulus to be determined.

Subtle heterogeneity in cellular responses can be discerned in this way. The most widely implemented application of JC-1 is for detection of mitochondrial depolarization occurring in the early stages of apoptosis (1, 2, 3, 4, 5).

For this technical note mitochondrial depolarization was induced by applying different concentrations of Antimycin A. Antimycin A is a mitochondrial inhibitor that is involved in the energy-coupling site of the respiratory system. It works by inhibiting the flow of electrons from cytochrome b to cytochrome c₁. Antimycin was first discovered as a potent fungicide that was produced by a species of Streptomyces.
Researchers found that the toxic effects of Antimycin were a result of their inhibitory effects on mitochondrial respiration.

Material and Methods

Cell culture: A431 cells (human epidermoid carcinoma cells, ATCC No. CRL-1555) were kindly provided by Prof. Barbara Krammer, University of Salzburg, and maintained in culture in ‘standard medium’ (DMEM, Sigma, D-5671) supplemented with L-Glutamine, Na-Pyruvat, Penicillin/Streptomycin, HEPES and 5% heat inactivated fetal calf serum (FCS, PAA laboratories, Austria) in an atmosphere of 5% CO₂ and 37 °C. For treatment with JC-1 12500 cells/well were grown overnight in 96 well plates (GREINER, Cat. No.: 655090, 96 well plate, black, µClear, TC).

Staining: After overnight incubation culture medium was removed, 12500 cells were washed twice with Dulbecco’s phosphate buffered saline (DPBS). The staining solution was prepared by diluting the 2.5 mM JC-1 stock solution (Molecular Probes, T-3168) 1:75 with standard medium without fetal calf serum. The cells were treated with 100 µl of 5, 7.5 or 10 µM Antimycin A (Sigma, A-8674) solution in standard medium without FCS. Cells were incubated for 1 h in an atmosphere of 7.5% CO₂ and 37 °C.

After incubation Antimycin was removed and 50 µl of the staining solution were added and the cells incubated under shaking as stated above. The medium was removed and 200 µl of PBS plus 5% BSA were added. After further 5 minutes incubation time under shaking (as done before) PBS/BSA was removed and 100 µl PBS were added before performing the measurements.

Instruments: Tecan Ultra Evolution; Tecan GENios Pro; Tecan Safire.

Measurements: The green JC-1 signals were measured at Ex 485 nm (20 nm BW)/ Em 535 nm (25 nm BW), the red signals at Ex 535 nm (25 nm BW)/ Em 590 nm (20 nm BW). All Safire measurements were performed with 12 nm bandwidth. Single measurements (one measurement point per well) or multiple reads per well (circle, 4x4) were performed (see Figure 3 for geometrical view).

In addition the excitation and emission spectra of JC-1-cells treated with/without Antimycin A have been recorded with a Tecan Safire.

Results

Spectra: Excitation and emission spectra of JC-1 were recorded (Figure 2) with a Tecan Safire. The excitation peak was found at ~500 nm, the emission maximum at ~530 nm (monomer) and ~590 nm (aggregate). Based on these findings the excitation filter 485 nm (BW 20 nm) and the
emission filter 535 nm (BW 25 nm) for the green signal and the excitation filter 535 nm (BW 25 nm) and the emission filter 590 nm (BW 20 nm) for the red signal were selected for further measurements. All Safire measurements were performed with bandwidth 12 nm.

Figure 4: Measurement of JC-1 stained cells treated with different amounts of the mitochondrial inhibitor Antimycin. The ratio of red signal (RFU at 590 nm; live cells) to green signal (RFU at 535 nm, dead cells) of a measurement performed with a Tecan Ultra Evolution is shown.

Figure 5: The emission spectrum of JC-1 in cells treated with different concentrations of Antimycin is shown. The spectrum consists of two peaks: The fluorescence peak at about 590 nm is of the J-aggregate; the peak at about 530 nm comes from the monomer of JC-1.

### Discussion

JC-1 is a fluorescence mitochondrial potential sensor, which is mainly used for FACS analysis for detection of mitochondrial depolarization occurring during the early stages of apoptosis. However, this technical note shows that JC-1 could also be used in a microplate fluorometer for a basic differentiation between live and dead cells. Using the bottom reading option, Tecan Ultra Evolution, Safire and GENios Pro are capable of detecting the effects of Antimycin on cells and distinguish between live and dead cells.

The fluorescence spectrum of JC-1 (Figure 2) within cells was measured with a Tecan Safire. Two emission peaks could be detected which are caused by the two different conformations of JC-1. A so-called J-aggregate is formed in the mitochondria of living cells mainly depending on the membrane potential of the mitochondria. The J-aggregate has its emission maximum at about 590 nm. The monomer can be found in cells when the mitochondrial potential decreases (e.g. in case of apoptosis) or also in...
dead cells. Its emission maximum is at about 530 nm.

Antimycin inhibits the function of mitochondria and induces cell death. Due to this effect the conformation of JC-1 changes from J-aggregate to monomer. The read signal (590 nm) of the J-aggregate therefore decreases and the green signal (530 nm) increases. Figure 4 shows the decreasing red/green signal rate with increasing Antimycin concentration. Comparing the negative control (no Antimycin added) with the Antimycin treated cells the ratio significantly decreases. However, the system is not sensitive enough to resolve subtle differences between the single Antimycin concentration steps.

Figure 5 again shows the effect of Antimycin on mitochondria. The emission spectra of JC-1 clearly show the conformational change described above.

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Literature


(3) Salvioli, S., A. Ardizzoni, C. Franceschi and A. Cossarizza: JC-1, but not DiOC₆(3) or rhodamine 123, is a reliable fluorescent probe to assess ΔΨ changes in intact cells: implications for studies on mitochondrial function during apoptosis. FEBS letters 1997, 411: 77 - 82.


Glossary

BSA Bovine Serum Albumin
BW Bandwidth
DMEM Dulbecco’s Modified Eagle’s Medium
DPBS Dulbecco’s phosphate buffered saline
FACS Fluorescence activated cell sorting
FCS Fetal calf serum
JC-1 5’, 6’, 6”-tetrachloro-1’, 3’, 3”- tetraethyl-benzimidazolcarbocyanine iodide
Mrpw Multiple reads per well
PBS Phosphate buffered saline