



Characterization of fluorescent DCFH₂-DA probe to use in photoinduced oxidation assays: ¹O₂ contributes to DCF formation.

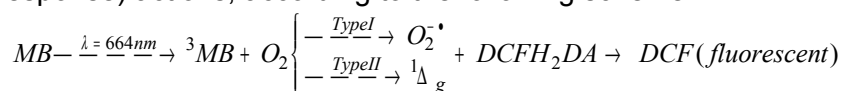
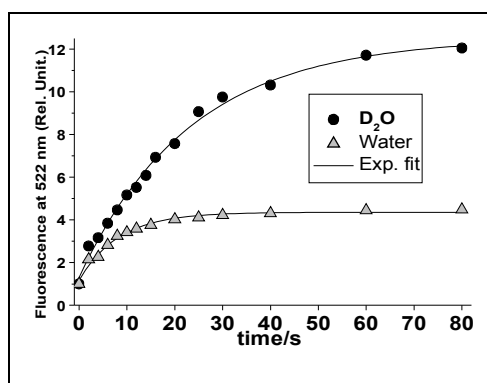
NA Daghasanli^{1*}, R Itri^{1}, MS Baptista^{2***}**

¹Inst. Física (IF-USP); ²Inst. Química (IQ-USP)
*nasser@if.usp.br; **itri@if.usp.br; ***baptista@iq.usp.br

DCFH₂-DA (2',7'-dichlorofluorescein diacetate) is a probe that has been extensively used to monitor oxidation in biological systems, and it is a classical indicator of reactive species formation in vitro [1], forming DCF (2',7'-dichlorofluorescein), a highly fluorescent ($\lambda_{max} = 522 \text{ nm}$) product when oxidized. On the other hand, the use of the probe to detect ¹O₂ was questioned [2]. Methylene blue (MB) is a photosensitizer that releases reactive species when irradiated, including ¹O₂ and radicals [3]. In aqueous solution, MB molecules are in monomer/dimer equilibrium. In the presence of SDS detergent a preferential MB monomeric (SDS>20 μ M), which that forms ¹O₂, or dimeric (SDS<3 μ M), which that forms O₂⁻, form takes place.

In this work we measured the reactive species released by MB in vitro with DCFH₂-DA /DCF fluorescent and phosphorescent assays. 100 nM of DCFH₂-DA was added to 3 μ M of MB solutions, in water and in deuterated water (D₂O) at pH (pD) 7. The solutions were irradiated under visible diode laser light (664 nm, 50 mW) in a stirred and air equilibrated 1 cm cuvette, and the fluorescence (F(t)) of samples was measured. ¹O₂ phosphorescence at 1270 nm was measured with NIR spectrometer by MB excitation in the presence of SDS.

Figure shows the kinetics of fluorescence increasing (at 522 nm), due to DCF formation, in water (Δ) and in D₂O (\bullet) as a function of irradiation time ($F(t) = F_{max}[1 - \beta e^{-t/k}]$). The rates of DCF fluorescent formation (V_F) were calculated according to an exponential approximation ($V_F \equiv \frac{d}{dt} F(t) = \frac{\beta F_{max}}{k}$). The results show that the V_F in D₂O is 3.6 greater than in water. The phosphorescence of ¹O₂ in presence of monomer was 25% greater than in water, and 87% greater than in dimer. These results indicate that ¹O₂ contributes to DCF formation. We can conclude that the DCFH₂-DA may suffer oxidation by O₂⁻ (a classical response) and ¹O₂ (an unexpected response) actions, according to the following scheme.



- 1- A Gomes, E Fernandes, JLFC Lima. J. Biochem. Biophys. Methods 65:45–80, 2005.
- 2- P Bilski, AG Belanger, CF Chignell. Free Rad. Biol. Med. 33(7): 938–946, 2002.



XI Congresso Brasileiro de Física Médica

<http://www.abfm.org.br/rp2006/index.asp>

14 a 17 de Junho de 2006 - Ribeirão Preto - SP

3- JP Tardivo, A Del Giglio, CS de Oliveira, DS Gabrielli, HC Junqueira, DB Tada, D Severino, RF Turchiello, MS Baptista. Photodiagnosis and Photodynamic Therapy, 2(3):165-238, 2005.