Application of probes ^{77}Se NMR and NADPH-FAD fluorescence at Thioredoxin 1 and Reductase Thioredoxin 1 from Saccharomyces cerevisiae.

Antunes H.A.[1], Almeida FC[2], Netto LE [3], Lima LM [4], Valente AP [2] .

Centro Nacional de Ressonância Magnética Nucelar Jiri Jonas, Instituo de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro-RJ, Brasil.

The components of the Thioredoxin system are Thioredoxin (Trx) and Thioredoxin Reductase (Trr) enzymes. We performed two strategies to probe the active site of Trx1 and Trr1 isoforms: ^{77}Se NMR Spectroscopy and Fluorescence Ressonance Energy Transfer (FRET) between FAD and NADPH cofactors. The strategy was developed to label of the Trx1 active site with Selenocysteine. The SDS-PAGE showed a high expression of Trx1 in 22 hours after of induction with IPTG in presence of Selenocysteine. Trx1 was purified by exclusion molecular chromatography and analyzed by MALDI-TOF spectrometry. The ^{77}Se-^{1}H NMR spectroscopy for reduced Trx1 revealed three ressonance lines for $^{1}H_{\beta 1}$ of Selenocysteine 30 and 33, between 1.6 and 2,0 ppm. The 2D-HMQC show the ^{77}Se ressonance signal at 378 ppm, coupled with $^{1}H_{\beta 1}$ and $^{1}H_{\beta 2}$ lines. The 1D-HMQC for oxidized Trx1 revealed the only one broad resonance in 2.6 ppm relative to the $^{1}H_{\beta 1}$ prótons. The 2D-HMQC spectrum of oxidized protein shows the one higher chemical shift (1038 ppm) of ^{77}Se if compared with reduced state. The FRET between NADPH-FAD cofactors at Trr1 was monitored through NADPH fluorescence (λ_{exc}=340nm) and scanning the spectrum between 400 and 600nm. The fluorescence spectrum of NADPH and FAD cofactors under reduced conditions show energu transfer between cofactors. It was´nt observed to oxidized Trr1 suggesting that Trr1 change the conformation according to redox potential of medium. Therefore the FRET and ^{77}Se NMR spectroscopy can be used as probes to provide insights at enzymatic mechanisms from thioredoxin oxidoreduction system.