

SITE-DIRECTED SPIN LABELING STUDIES OF E_cDHODH/VESICLES INTERACTION

Couto, S. G.¹, Nonato, M. C.², Costa-Filho, A. J.¹

1- Grupo de Biofísica Molecular Sérgio Mascarenhas, Instituto de Física de São Carlos, Universidade de São Paulo

2- Laboratório de Cristalografia de Proteínas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo

Introduction: Electron Spin Resonance (ESR) has been experiencing a moment of resurgence in structural studies of protein and lipid-protein interactions since the introduction of site-directed spin labeling (SDSL) methodology, where a nitroxide side chain is selectively introduced at specific sites using site-directed mutagenesis. In this work, we present results obtained from SDSL studies on the interaction of dihydroorotate dehydrogenase (DHODH) with mixed vesicles. DHODH catalyzes the fourth step in the *de novo* pyrimidine nucleotide synthesis pathway. Results: ESR spectra of labeled phospholipids introduced in vesicles show that the protein binds to mixed as well as pure detergent micelles. A second less-immobilized component is clearly observed in the spectra of phospholipids chain labels 5- and 10-PC, which is absent when other labels, such as headgroup label DPPTC and 16-PC, are used. The appearance of such component is attributed to the formation of a defect in the micelle hydrophobic region. SDSL studies were performed on residues Y2, F5, H19, and F21, which are located at the N-terminal extension of the protein. Their ESR spectra were characterized in terms of protein local backbone fluctuations. Conclusions: ESR and SDSL techniques were used to investigate DHODH/vesicles interactions. To the best of our knowledge, this is the first report showing direct evidences concerning the binding of class 2 DHODH to membrane systems and its implication in protein function. Also, this is the first site-directed spin labeling study fully developed in Brazil. Acknowledgments: FAPESP, CNPq. Keywords: ESR, site-directed spin labeling, lipid-protein interactions.