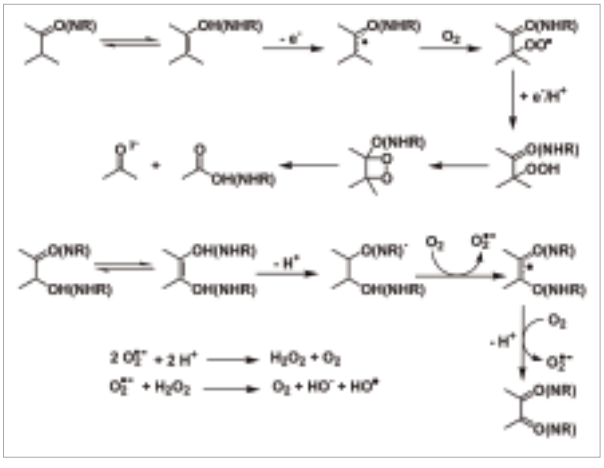
REDOX AND CARBONYL STRESS ASSOCIATED WITH ENDOGENOUS ALFA-AMINOKETONES AND BETA-KETOACIDS: MECHANISMS AND BIOMARKERS

Etelvino José Henriques BECHARA

Chemistry Institute / University of São Paulo (USP)



Radical acetylation of L-histidine and 2'-deoxyguanosine coupled to the reaction of peroxynitrite with diacetyl in aerated aqueous medium

Oxygen and nitric oxide are known to act normally as metabolic partners in biological events and any imbalance in this cooperative relationship results in oxidative and nitrosative damage to biomolecules and organelles, threatening the cell's life. Our research proposal focuses on the pro-oxidative and acylation properties of amino acid metabolites, particularly α-aminocarbonyls and β-ketoacids that accumulate in the tissues of individuals carrying metabolic errors. We aim to study: (i) 5-aminolevulinic acid (ALA), a heme precursor elevated in intermittent acute porphyria and lead poisoning; (ii) aminoacetone (AA), a threonine and glycine catabolite accumulated in diabetes, threoninemia, and *cri-du-chat*; (iv) succinylacetone, a β-diketone produced at high levels from tyrosine in tyrosinosis, where it leads indirectly to ALA overload; and (iv) 2- methylacetoacetate (MAA), an isoleucine catabolite abundant in isoleucinemia carriers. These metabolites undergo aerobic oxidation yielding cytotoxic and genotoxic species such as radicals, peroxides, triplet species and α-oxoaldehydes. The latter compounds encompass 4,5-dioxovaleric acid (DOVA), linked to ALA and SA accumulation, and methylglyoxal (MG), derived from AA and SA. In the next four years we plan to continue investigating the chemical mechanisms of ALA, AA, SA, and MA oxidation in aerated medium, triggered by metals, peroxynitrite, hemeprotein/H_2O_2 or hypochloride, as well as the damaging effects of their intermediates and final products – DOVA, MG, and biacetyl – on cell cultures, rats and humans. In parallel, we will developed analytical methods by CE/MS/MS for detecting these metabolites in biological samples and other biomarkers of carbonyl stress.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Aminoacetone (AA) was shown to induce apoptosis and necrosis in insulin-producing RINm5f cells that can be attributed to copper-catalyzed AA-generated superoxide radical, H_2O_2, and MG. AA-triggered cell death, as expected, is prevented by treatment with the antioxidant *N*-acetylcysteine (NAC). Flow cytometry studies indicated that the AA cytotoxicity to RINm5f implicates alterations in calcium fluxes, mitochondrial membrane polarization, and expression of BAX, BCL-2, and BCL-XL apoptotic proteins. Similar experiments with pancreas islets will be performed in an attempt to shed light on the role of those putative endogenous toxicants in diabetes. Regarding our work on ALA-related porphyrias (AIP, tyrosinosis, and plumbism), we have succeeded to establish a reliable experimental model based on i.p. injection of SA in rats, a strong ALA dehydratase inhibitor. Increased production of ALA, decrements in porphyrins, elevated indices of oxidative stress, and histological data are consistent with long reported mitochondrial changes observed in liver biopsies of AIP patients. In parallel, the hypothesis of lead-induced adolescent anti-social behavior is underway in Bauru (SP). Considering that ALA is an endogenous precursor of photosensitizing porphyrins, a by-product of our project is the formulation of a cream containing ALA methyl ester that was successfully used in photodynamic therapy of feline squamous cell carcinoma. Finally, our hypothesis on possible involvement of acyl radicals, generated by the reaction of α-dicarbonyls with peroxynitrite, in protein and DNA damage during nitrosative and carbonyl stress, seems indeed promising, once we verified authentic L-amino acids and 2′-deoxyguanosine acetylation by the diacetyl/peroxynitrite system. In the course of these investigations we have established CE/MSMS methodologies to analyze the metabolites mentioned here and glutathione-derived biomarkers of oxidative stress.

MAIN PUBLICATIONS

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Etelvino José Henriques BECHARA

Instituto de Química
Universidade de São Paulo (USP)
Av. Prof. Lineu Prestes, 748, bloco 10 superior,
sala 1074 – Cidade Universitária
CEP 05508-900 – São Paulo, SP – Brasil

+55-11-3091-3869
ebechara@iq.usp.br