Methylglyoxal Reaction with Peroxynitrite Coupled to Lysine Acetylation

Massari, J., Tokikawa, R., Assunção, N.A., Zanolli, L., Tavares, M.F.M., Bechara, E.J.H.

Instituto de Química, Universidade de São Paulo, São Paulo, Brazil

Methylglyoxal (MG) is reportedly produced in excess by triose phosphates and aminoacetone in some disorders, particularly in diabetes. In turn, the peroxynitrite anion ($ONOO^-$) is a potent oxidant and nucleophile formed in vivo by a diffusion controlled reaction between $O_2^{\bullet-}$ and •NO. Here, we study the nucleophilic addition of $ONOO^-$ to MG in phosphate buffer yielding a nitrosoperoxoadduct, whose homolysis leads to acetyl radical and ultimately acetate ions. Using stopped-flow UV spectrophotometry, the rate of $ONOO^-$ decay in the presence of MG (k_2 = 1.0×10^5 $M^{-1}s^{-1}$), pH 7.2, at 25ºC, was found to be faster than that reported for monocarbonyl substrates ($k_2 < 10^3 M^{-1}s^{-1}$) as well as for diacetyl (k_2= 1.0×10^4 $M^{-1}s^{-1}$), CO_2 ($k_2 = 5.8\times10^4$ $M^{-1}s^{-1}$) (Massari et al., CRT 2008) and possibly glyoxal (k_{2app} = $3.3x10^2$ $M^{-1}s^{-1}$; $k_2 > 10^5$ $M^{-1}s^{-1}$). The MG reaction displays a pH profile with a shoulder at pH 7.2, coincident with the pKa values of both ONOOH and $H_2PO_4^-$. EPR spin trapping studies with MNP revealed acetyl radical adduct (a_N = 0.83 mT) formation, whose triplet signal intensity responds to both [$ONOO^-$] and [MG]. This adduct could also be observed by replacing $ONOO^-$ for H_2O_2, although at much lower yields. Stoichiometric amounts of formate and acetate were detected by capillary electrophoresis in the spent reaction mixture. CE-MS analysis of the reaction mixture containing L-Lysine revealed a MSMS spectrum attributable to a N-ε-acetyl-lysine adduct. These data support our hypothesis that acetylation of protein residues might occur in vivo by a radical route under pathological conditions where $ONOO^-/H_2O_2$ and a-dicarbonyls accumulate, as seems to be the case of inflammatory processes. Support: FAPESP, CNPq, INCT Redoxoma.