

Oxidation of free, peptide and protein tryptophan residues mediated by AAPH-derived free radicals: role of alkoxy and peroxy radicals

Por: Fuentes-Lemus, E (Fuentes-Lemus, E.)^[1]; Dorta, E (Dorta, E.)^[1]; Escobar, E (Escobar, E.)^[1]; Aspee, A (Aspee, A.)^[2]; Pino, E (Pino, E.)^[2]; Abasq, ML (Abasq, M. L.)^[3]; Speisky, H (Speisky, H.)^[4]; Silva, E (Silva, E.)^[5]; Lissi, E (Lissi, E.)^[2]; Davies, MJ (Davies, M. J.)^[6] ...Más

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Resumen

The oxidation of tryptophan (Trp) residues, mediated by peroxy radicals (ROO.), follows a complex mechanism involving free radical intermediates, and short chain reactions. The reactivity of Trp towards ROO. should be strongly affected by its inclusion in peptides and proteins. To examine the latter, we investigated (by fluorescence) the kinetic of the consumption of free, peptide-and protein-Trp residues towards AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride)-derived free radicals. Interestingly, the initial consumption rates (R-i) were only slightly influenced by the inclusion of Trp in small peptides and proteins (human serum albumin and human superoxide dismutase).

Depending on the Trp concentration, the R-i versus Trp concentration ([Trp]) plots showed three regions. At low Trp concentrations (1-10 μ M), a linear dependence was observed between R-i and [Trp]; at intermediate Trp concentrations (10-50 μ M), the values of R-i were nearly constant; and at high Trp concentrations (50 μ M to 1 mM), a slower increase of R-i than expected for chain reactions. Similar behavior was detected for all three systems (free Trp, and Trp in peptides and proteins). For the first time we are showing that alkoxy radicals, formed from self-reaction of ROO., are responsible of the Trp oxidation at low concentrations, while at high Trp concentrations, a mixture of peroxy and alkoxy radicals are involved in the oxidation of Trp residues.

Palabras clave

KeyWords Plus: AMINO-ACID-RESIDUES; SUPEROXIDE-DISMUTASE; SOY PROTEIN; INACTIVATION; OXYGEN; ANTIOXIDANTS; LYSOZYME; KINETICS; DAMAGE; DIHYDROCHLORIDE

Información del autor

Dirección para petición de copias: Lopez-Alarcon, C (autor para petición de copias)



Pontificia Univ Catolica Chile, Fac Quim, Dept Farm, Santiago, Chile.

Direcciones:

- + [1] Pontificia Univ Catolica Chile, Fac Quim, Dept Farm, Santiago, Chile
- + [2] Univ Santiago Chile, Fac Quim & Biol, Santiago, Chile
- + [3] Univ Rennes 1, Fac Sci Pharmaceut & Biol, Rennes, France
- + [4] Univ Chile, INTA, Santiago, Chile
- + [5] Pontificia Univ Catolica Chile, Dept Quim Fis, Fac Quim, Alameda 340, Santiago, Chile
- + [6] Univ Copenhagen, Panum Inst, Dept Biomed Sci, DK-1168 Copenhagen, Denmark

Direcciones de correo electrónico: clopezr@uc.cl

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ROYAL SOC CHEMISTRY, THOMAS GRAHAM HOUSE, SCIENCE PARK, MILTON RD,
CAMBRIDGE CB4 0WF, CAMBS, ENGLAND

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