Chemiluminescent microspheres for measuring reactive oxygen species (ROS) in phagocytosis

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Analogues of Cypridina luciferin, namely, CLA, MCLA and FCLA were bound to polymer microspheres for the purpose of measuring ROS released into phagosomes during phagocytosis. CLA and MCLA were absorbed into or adsorbed on microspheres (ca. 1µm) of a methyl methacrylate copolymer, while FCLA was covalently bound to microspheres (ca. 2µm) of a glycidyl methacrylate copolymer derivative through amide group that was formed by the condensation reaction of an amino group in the polymer with a carboxyl group in FCLA. ROS were generated by O2/hypoxanthine/xanthine oxidase (O2−), KO2 (O2−), NDPO2 (1O2), H2O2/MPO/Cl−, or H2O2/Fe2+ (HO·). Desorption of CLA or MCLA from microspheres impregnated with them was less than 5% at pH 7.4 (PBS) and pH 5.2 (citrate buffer). The release of FCLA from FCLA-bound polymer microspheres was substantially negligible. MCLA-impregnated microspheres and FCLA-bound microspheres emitted stronger chemiluminescence than commercially available ABEI-bound polymer microspheres. The HO· radical generated in H2O2/Fe2+ system elicited stronger luminescence from FCLA-bound microsphere than O2− liberated from KO2. 1O2 from NDPO2 elicited insufficient chemiluminescence from any kind of these microspheres, whereas it produced strong chemiluminescence with the solution of every kind of these Cypridina luciferin analogues. As a conclusion, polymer microsphere impregnated with MCLA and FCLA-bound polymer microsphere are promising probes for measuring O2− and HO· released into phagosomes in phagocytosis.