CHEMILUMINESCENCE IN ACTIVATED HUMAN NEUTROPHILS: Role of Buffers and Scavengers

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Abstract—Human neutrophils (PMNs) suspended in Hanks' balanced salt solution (HBSS), which are stimulated either by polycation-opsonized streptococci or by phorbol myristate acetate (PMA), generate nonamplified (CL), luminol-dependent (LDCL), and lucigenin-dependent chemiluminescence (LUCDCL). Treatment of activated PMNs with azide yielded a very intense CL response, but only a small LDCL or LUCDCL responses, when horse radish peroxidase (HRP) was added. Both CL and LDCL depend on the generation of superoxide and on myeloperoxidase (MPO). Treatment of PMNs with azide followed either by dimethylthiourea (DMTU), deferoxamine, EDTA, or detapac generated very little CL upon addition of HRP, suggesting that CL is the result of the interaction among H2O2, a peroxidase, and trace metals. In a cell-free system practically no CL was generated when H2O2 was mixed with HRP in distilled water (DW). On the other hand significant CL was generated when either HBSS or RPMI media was employed. In both cases CL was markedly depressed either by deferoxamine or by EDTA, suggesting that these media might be contaminated by trace metals, which catalyzed a Fenton-driven reaction. Both HEPES and Tris buffers, when added to DW, failed to support significant HRP-induced CL. Nitrilotriacetic acid (NTA) chelates of Mn2+, Fe2+, Cu2+, and Co2+ very markedly enhanced CL induced by mixtures of H2O2 and HRP when distilled water was the supporting medium. Both HEPES and Tris buffer when added to DW strongly quenched NTA-metal-catalyzed CL. None of the NTA-metal chelates could boost CL generation by activated PMNs, because the salts in HBSS and RPMI interfered with the activity of the added metals. CL and LDCL of activated PMNs was enhanced by aminotriazole, but strongly inhibited by diphenylene iodonium (an inhibitor of NADPH oxidase) by azide, sodium cyanide (CN), cimetidine, histidine, benzoate, DMTU and moderately by superoxide dismutase (SOD) and by deferoxamine.

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LUCDCL was markedly inhibited only by SOD but was boosted by CN. Taken together, it is suggested that CL generated by stimulated PMNs might be the result of the interactions among, NADPH oxidase, (inhibitable by diphenylene iodonium), MPO (inhibitable by sodium azide), H$_2$O$_2$ probably of intracellular origin (inhibitable by DMTU but not by catalase), and trace metals that contaminate salt solutions. The nature of the salt solutions employed to measure CL in activated PMNs is critical.

INTRODUCTION

It is well established that neutrophils (PMNs), which are stimulated by a variety of both soluble and particulate agonists generate a series of oxygen-derived species (O$_2$, H$_2$O$_2$, OH$^\cdot$ and $^{1}$O$_2$) that can be measured by a variety of techniques (1–6). Concomitant with the generation of oxygen-derived species, stimulated PMNs also emit light (chemiluminescence, CL), which can be monitored either by scintillation counters or by a variety of luminometers (7–14). Light emission can be markedly amplified either by luminol (luminol-dependent chemiluminescence, LDCL) (7–13) or by lucigenin (lucigenin-dependent chemiluminescence LUCDCL) (12). While LDCL probably measures a mixture of oxygen-derived species, LUCDCL is believed to specifically monitor the generation of superoxide. This vast field of research has been reviewed elsewhere (13, 15).

In addition to the standard stimulators of superoxide and CL generation by PMNs and macrophages regularly employed (antibody-opsonized particles, chemotactic peptides, phorbole esters), we have recently introduced a variety of cationic polyelectrolytes (poly-L-arginine, poly-L-histidine, histone) as potent stimulators of the respiratory burst in mammalian PMNs (16–22).

A strong relationship between the respiratory burst in neutrophils and the phenomenon of chemiluminescence has been established (13). The findings that either azide-treated normal PMNs or PMNs from patients with MPO deficiency failed to generate luminol-dependent chemiluminescence suggested a link between the respiratory burst, the phenomenon of LDCL, and the MPO–H$_2$O$_2$–halide system (10). In a more recent study, a critical analysis of the controversial issues connected with the employment of chemiluminescence to evaluate the role of discrete oxygen-derived species in light emission by activated PMNs has been reported (15). These authors came to the conclusion that “luminol-dependent chemiluminescence gives, at present, very little ability to discriminate between individual oxygen species.” Furthermore, “luminol-dependent chemiluminescence used in biological systems is extremely prone to many interferences, which are very difficult to control.” Thus, although measurement of chemiluminescence generation by PMNs is very simple to perform, many investigators of neutrophil activation are reluctant to employ chemiluminescence techniques to evaluate the nature of the oxygen-derived species that are responsible for light emission.