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EXCITATION OF INDOLE ANALOGS BY PHAGOCYTOSING LEUKOCYTES

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SUMMARY

The system suspended with phagocytosing leukocytes and related system produce weak light which could be greatly amplified by indole analogs with plain fatty acids at 3 position. Main emitting species in indole-3-acetic acid or indole-3-propionic acid-sensitized system was analyzed spectrometrically in the dark and ascribed to the transition of an excited indole compound in triplet state to its ground state. Such an excited species would be generated by the oxidative way of the indole analogs but not through the dioxetane structure of 2 and 3 positions on indole ring.

INTRODUCTION

It has been reported that phagocytosing leukocytes and artificially activated leukocytes produce the weak light which could be detected by a scintillation counter (1-8). In spite of no precise characterization of $^1O_2$ in these systems, an emitting species is considered to be involved in $^1O_2$ (1,2). If appreciable amounts of $^1O_2$ is generated in such systems, $^1O_2$ would attack indole analogs with the formation of excited acetophenone analogs which in turn emit fluorescence (9, 10).

The present report describes that indole analogs (IAA and IPA) are excited by phagocytosing leukocytes (OZ-system) and

Abbreviations: IAA, indole-3-acetic acid; IPA, indole-3-propionic acid, IBA, indole-3-butyric acid; OZ, opsonized zymosan; PMA, phorbol myristate acetate; $^1O_2$, singlet oxygen; L-Lox, linoleate-lipoxygenase; DBAS, 9, 10-dibromoanthracene sulfonate; O_2^-, superoxide anion

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artificially activated leukocytes (PMA-system), probably not through $^1O_2$ derived way.

MATERIALS AND METHODS

Leukocytes were prepared from peripheral human blood by a modification of the method of Curnutte and Babior (11). Leukocyte-suspension was kept for 10 hours in an ice bath before the experiments. OZ-system was consisted of 4mg of OZ (4) and 10^7 cells in 4ml of Krebs-Ringer phosphate buffer (pH 7.4) containing 0.2% glucose and 0.2% bovine serum albumin. PMA-system was essentially the same as OZ-system, save that 4μg of PMA was added instead of OZ. The reaction was initiated by the addition of OZ or PMA. Chemiluminescence was measured in a Beckman Model LS-200B liquid scintillation counter (with the coincidence circuit off) at 22°C (12). During the luminescent measurement, the reaction mixture in a siliconized vial was gently agitated for 20 second and immediatly placed in the chamber for 30 second-reading, then remixed before each subsequent counts. An average of initial three counts of control system excluding OZ and PMA was used for back ground counts. Luminescence intensity (counts per 30 second) was corrected for the back ground counts. The incubation mixture for the determination of D_2O effect was prepared by the method described previously, save that leukocytes suspended in a small volume of Krebs-Ringer phosphate buffer were used instead of enzyme source (12). Emission spectra (luminescence spectra) were taken by a spectrometer (13). $O_2^{\cdot-}$ accumulation in the system was measured by the reduction of exogeneously added ferricytochrome c (5) at 22°C.

RESULTS AND DISCUSSION

When IAA or IPA at 1.5 mM was added to OZ-system or PMA system, it was found to increase obviously the luminescence intensity without significant change in luminescence curves VS time. The maximal rate of $O_2^{\cdot-}$ accumulation in OZ- or PMA-system was suppressed by the addition of IAA or IPA, suggesting the utilization of $O_2^{\cdot-}$ for the oxidation of the indole analogs. Some of these results are summarized in Table 1. To test the oxidizability of IAA in such systems, 200μM IAA containing a tracer dose of 3-indolyl-[2-^{14}C]-acetic acid was exposed to OZ-system for 30min at 22°C and the products were analyzed by thin layer chromatography (14). The radio-chemical assay and visualization of products on the thin layer plate indicated that 20% of IAA was degraded to unknown compounds but not to

TABLE I

EFFECT OF IAA OR IPA ON THE LUMINESCENCE AND O_2^- ACCUMULATION IN OZ-SYSTEM AND PMA-SYSTEM

System	peak intensity (counts/30s)	n moles/10^7 cells/min cytochrome c reduction (maximal rate)
OZ-system	60,135	5.8
OZ-system + IAA	222,499	4.7
OZ-system + IPA	330,742	3.9
PMA-system	19,809	12.7
PMA-system + IAA	154,510	11.9
PMA-system + IPA	309,020	9.4

$^1O_2$-derived products (o-formylamino acetophenone and o-formylaminobenzoylacetic acid, produced by the cleavage of the dioxetane structure at 2 and 3 positions on indole ring (14)).

As shown in Table II, substituents at the 3 position on indole ring affect indole-sensitized luminescence in OZ-system. Indole analogs with plain fatty acids and N-inactivated alanine at the 3 position on indole ring were powerful sensitizers, while those with dimethyl acetic, lactic and alanine in the same position were less effective or inhibitory. These views are similar to the indole-sensitized luminescences in L-Lox-system, but differ from the latter in the order of magnitude of sensitization; i.e., IAA > IPA > IBA for L-Lox-system (14). Furtheremore, in contrast to IAA-sensitized luminescence in L-Lox-system, the replacement of H_2O in the IAA- OZ-system with D_2O decreased the maximal light intensity with increasing amounts of

TABLE II

EFFECT OF INDOLE ANALOGS ON THE LUMINESCENCE IN OZ-SYSTEM

Indole-3-R* (R)	peak intensity (counts/30s)	relative peak intensity
None	75,400	1.0
-COOH	157,500	2.1
$-CH_2COOH$	358,400	4.8
$-C(CH_3)_2COOH$	76,300	1.0
$-CH_2CH_2COOH$	533,200	7.1
$-CH_2CH(NH_2)COOH(L)$	42,200	0.6
$-CH_2CH(NHCOCH_3)COOH(L)$	221,100	2.9
$-CH_2CH(OH)COOH(DL)$	59,900	0.8
$-CH_2CH_2CH_2COOH$	472,900	6.3
$-CH_2CH_2NH_2$	119,100	1.6

* Substrate concentration at 1.5mM

D_2O (Fig. 1). However, the emission spectra of IAA- (or IPA)-OZ-system and IAA- PMA-system in the region between 400 and 525nm were almost identical to each other and to that obtained with L-Lox-system in presence of IAA (14)(Fig. 2). Such emission spectra appear to be quite similar to those reported for photoexcited indole and IAA phosphorescences ($\pi - \pi^*$) in EPA at 77°K (15,16), assuming red-sift (35nm) via a solvent effect. Thus, the excited species produced in the present systems is likely to be an excited indole compound in triplet state. Recently, excitation of IAA or DBAS in L-Lox-system has been demonstrated to be due to the transfer of the energy of excited carbonyl to indole ring (without degradation of IAA) or to DBAS

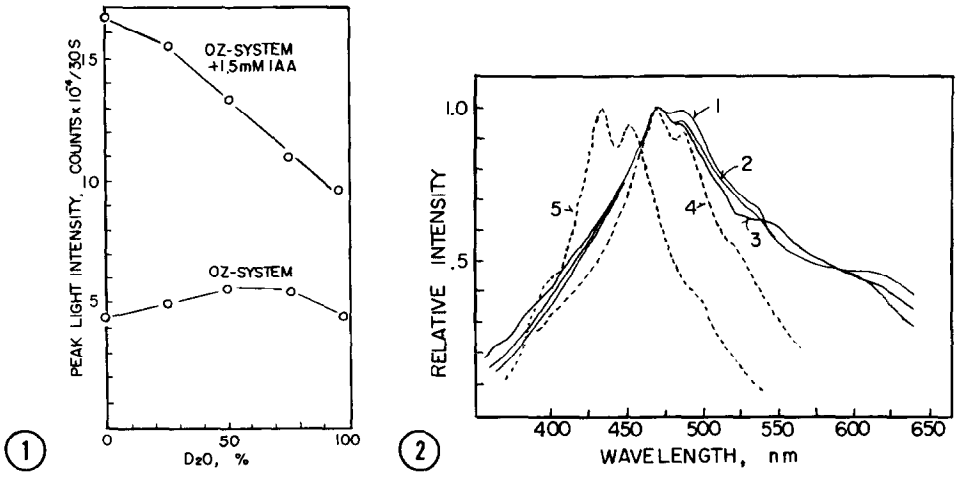

Figure 1. Effect of D_2O on luminescence of OZ-system in the presence or absence of IAA

Figure 2. Emission spectra of OZ- and PMA-systems in the presence of IAA or IPA in comparison with other indole-sensitized systems. Systems : 1, OZ-system + 1.5mM IAA; 2, OZ-system + 1.5mM IPA; 3, PMA-system + 1.5mM IAA; 4, L-Lox-system + 1.5mM IAA (after Nakano and Sugioka (14)); 5, Photoexcited indole in EPA at 77°K (after Fujimori (16)).

(12,14). OZ- and PMA-systems, however, do not produce energetically high species which can excit DBAS (17). It seems likely therefore that in OZ- and PMA- systems indole analogs with plain fatty acids at 3 position could be directly excited by some oxidative way, but not through the oxidative cleavage of indole ring. Even though the excitation mechanism is not clarified at the present, IAA or IPA is useful sensitizer for amplifying the weak luminescence produced by phagocytosing leukocytes.

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