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# THE CHEMILUMINESCENCE FROM THE REACTION OF HEMOPROTEINS WITH INORGANIC PEROXIDES

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Chemiluminescence from hemoproteins oxidation are composed of two components: the dimol emission of the singlet oxygen and the excited carbonyls, accompanied by the destruction of porphyrin rings.

The biological importance of hemoproteins is not inferior to DNA. The development of rapid and simple measurement for hemoproteins is certainly of great clinical and biological value.

It has been reported that in the chemiluminescent reactions of luminol and lipid peroxides, hemoproteins act as catalysts[1,2]. But the instability of luminol has also been noticed[3]. Furthermore, the pure organic peroxides are hard to come by.

We found that when hemin and hemorpoteins react with inorganic peroxides (sodium perborate or $H_2O_2$) in alkaline solution, low-level chemiluminescences could be detected (Fig.1,2).

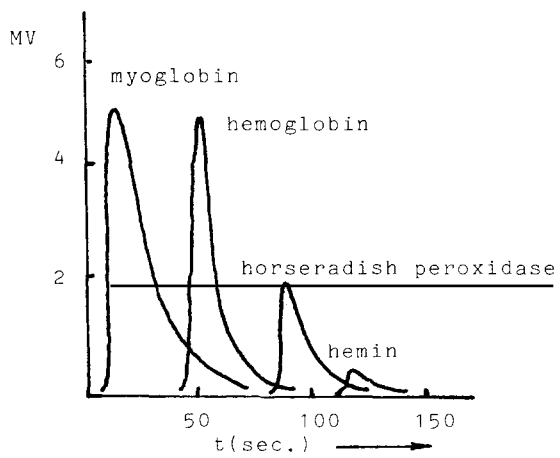


FIGURE 1
Chemiluminescent emission dynamic curves of myoglobin, hemoglobin, horseradish peroxidase and hemin.

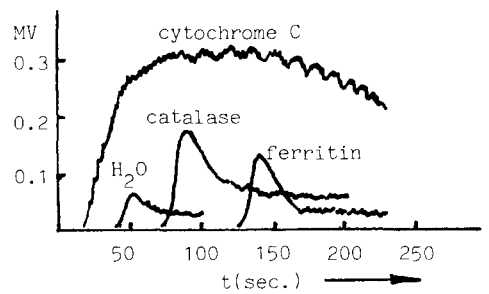


FIGURE 2
Chemiluminescent emission dynamic curves of cytochrome C, catalase, ferritin and water blank.

Measured with our home-made luminometer, the luminosity sequence of hemoproteins and control materials was as follows (the integral time was set to 100 seconds):

myoglobin > hemoglobin > horseradish peroxidase > cytochrome C > hemin > catalase > ferritin > water blank > $FeSO_4$ > $FeCl_3$ > hematoporphyrin + iron ion

Determined by OMA-2 multichannel spectroscopy and cutting filters, two bands appeared in the emission spectra: a red band with a peak around 630 nm and a blue band with a peak around 440 nm (Fig.3).

The red band must be the dimol emission of singlet oxygen, as these chemiluminescences could be strongly quenched by β-carotene. The blue band is supposed to be the luminescence from the excited carbonyls, which might be produced through the Dioxetane mechanism[1].

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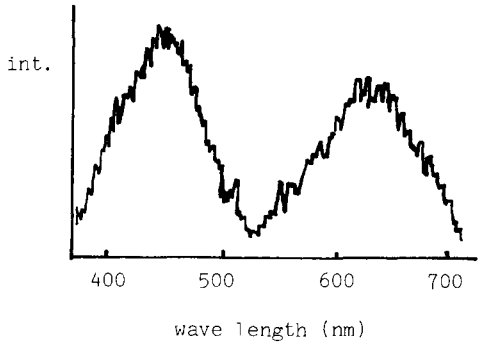


FIGURE 3
OMA-2 emission spectrum of myoglobin oxidation.

The scavengers of hydroxyl radicals could partially inhibit these chemiluminescences, indicating the participation of active oxygen other than the singlet oxygen.

The blank luminescence of sodium perborate in alkaline solution could also be quenched by β-carotene to great extent and inhibited by hydroxyl radical scavengers to some extent, so the singlet oxygen might partially come from the Arneson mechanism[4].

During oxidation the Soret bands of hemin and hemoproteins decreased gradually, representing the destruction of porphyrin rings. The excited carbonyls might come from porphyrins and their adjacent groups. The luminosity differences among various hemoproteins might account for the different amounts of excited carbonyls produced during oxidation.

The fact that different hemoproteins emitted light at different rates when they were oxidized, might be caused by the different exposure states of heme groups to solvents.

The chemiluminescence induced by the mixture of hematoporphyrin and iron ion (ferrous or ferric) was much weaker than those of hemin and hemoproteins, and there was no apparent porphyrin destruction during oxidation. The complex compound of ferriporphyrin must play a unique role in these chemiluminescences.

The ground state of iron ion is paramagnetic. On the 3d orbits there are spin-unpaired electrons, their spin-spin coupling is very weak. After the iron ions being complexed into porphyrins, their unpaired electrons are involved in the bigger rings conjugations and become highly delocalized, thus the reactivities of both iron and porphyrin are markedly enhanced. Therefore, heme and hematoporphyrin behaved quite differently during oxidation.

Good linear relationships between concentration and luminosity were obtained, both for myoglobin at ng level and for horseradish peroxidase at μg level (the correlation coeffient was 0.9926 and 0.9975, respectively). These chemiluminescences may be helpful to the search for new luminescent assays. For example, serum myoglobin determination is valuable to the rapid diagnosis of acute myocardial infarction[5]; and, if the luminometer is sensitive enough, the luminescent assay for horseradish peroxidase may be a safe substitute for the traditional ELISA, protecting the operator from contacting the highly carcinogenic substrate, i.e., the 3,3'-diaminobenzidine(DAB).

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