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An investigation into the reaction of hemin-catalysed luminol oxidation by peroxy compounds

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Abstract

The oxidation of luminol by organic peroxy compounds catalysed by hemin was studied. The influence of the reaction conditions and the structure of the oxidant on CL emissions was evaluated. It was found that the light intensity produced during the reaction of peroxy acids and diacyl peroxides was not substantially influenced by substitution. Peroxyesters and hydroperoxides behaved differently and their structure strongly affected the light yield. No light was detected from dialkyl peroxides under investigative conditions. The results imply that hydroperoxides and peroxy acids are intermediate in peroxyesters and diacyl peroxide reactions, respectively. In the case of peroxyesters, the rate of the hydrolysis step is light-yield determining. A mechanism was proposed to explain the behaviour of peroxy compounds toward luminol and hemin.

Keywords: Chemiluminescence; Peroxy compounds; Luminol; Hemin

1. Introduction

Analytical methods based on chemiluminescence detection have been extensively applied to various compounds. They are generally inexpensive and have many advantages, such as low detection limits, a wide linearity range and a short analysis time. Among many available procedures, those based on luminol reactions with hydrogen peroxide have gained great importance. There is less interest concerning peroxy compounds as oxidants in chemiluminescent reactions, although they have many practical applications as initiators of free radical reactions, organic synthesis reagents, and they are present in natural and industrial processes involving oxidation of organic compounds by oxygen. As far as we know, only a few papers relating to direct CL detection of peroxy compounds have been published [1–4], but knowledge about utilised reactions when hydrogen peroxide is replaced by its organic derivatives is still not satisfactory. On the other hand, the mechanism of luminol oxidation by H_2O_2 is well established [5–7] and is shown in Scheme 1.

It can be subdivided into two parts. First one is the path leading to an active intermediate formed from luminol monoanion and oxidant (reaction 1).

The hydroxy radical can be replaced by other organic or inorganic radicals, depending on catalyst and reaction conditions. When peroxidase (or complexes such as hemin) are present, oxo-iron(IV) porphyrin radical cation (oxene) [8,9] acts as the oxidant of luminol, as is shown in Scheme 2.

Reactions of peroxy acids and hydroperoxides with iron porphyrins were also widely studied [10–13] and are summarised in Scheme 3.

Rate constants of reaction involved in luminol oxidation are summarised in Table 1.

Luminol oxidation to diazaquinone is significantly affected by rate of reaction between catalyst and oxidant. In case of iron porphyrins, and peroxy compounds rates of reactions 12, 15 and 17 are appreciably different (Table 1). The fastest is oxidation by peroxyacids $10^4\ M^{-1}\ s^{-1}$. Hydrogen peroxide reacts about 600 times slower ($12\ M^{-1}\ s^{-1}$) and hydroperoxides are the less reactive oxidants ($4\ M^{-1}\ s^{-1}$). The subsequent reaction between oxidised catalyst and luminol (reactions 13 and 14) is faster than these three reactions so they are limiting the rate of formation of active intermediate.

The second part of the mechanism is addition of peroxide anion (reaction 5) to the diazaquinone IV. Subsequently, through an intramolecular substitution (reactions 7 and 8) intermediate compound VI is formed. This part is relatively simple when hydrogen peroxide is used. However with organic peroxides the

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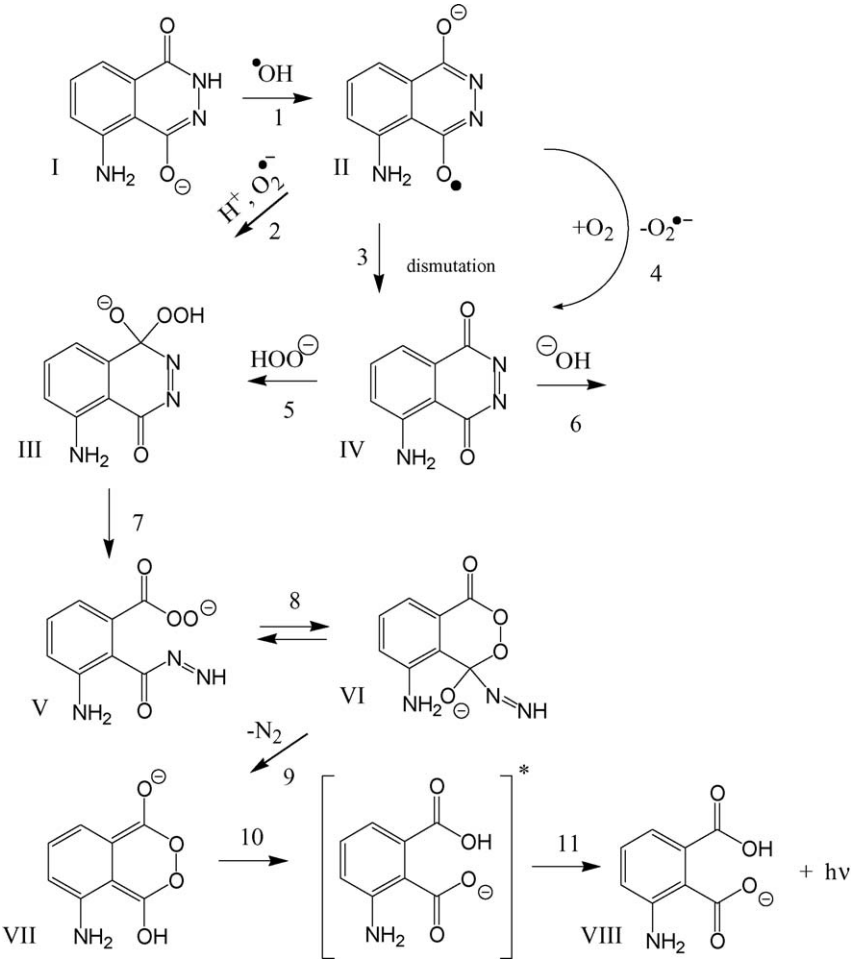


Scheme 1. Mechanism of luminol oxidation by hydrogen peroxide.

$$LFe_{(III)}^{+} + H_2O_2 \xrightarrow{12} H_2O + LFe_{(IV)}^{\bullet +}{=}O$$

$$I + LFe_{(IV)}^{\bullet +}{=}O \xrightarrow{13} II + LFe_{(IV)}^{+}\text{-}OH$$

$$I + LFe_{(IV)}^{+}\text{-}OH \xrightarrow{14} II + LFe_{(III)}^{+} + H_2O$$

Scheme 2. Hydrogen peroxide decomposition by iron porphyrins.

process is more complicated because all of organic peroxides have at least one carbon-peroxide bond, which of necessity must be broken if reaction 8 is to complete. This additional step may limit light yield. Another possible path leading to the intermediate III is the reaction between molecular oxygen and luminol

$$LFe_{(III)}^{+} + ROOH \xrightarrow{15} ROH + LFe_{(IV)}^{\bullet +}{=}O$$

$$ROOH + LFe_{(IV)}^{\bullet +}{=}O \xrightarrow{16} ROO^{\bullet} + LFe_{(III)}^{+} + H_2O$$

$$LFe_{(III)}^{+} + RCOOOH \xrightarrow{17} RCOOH + LFe_{(IV)}^{\bullet +}{=}O$$

$$ROO^{\bullet} + ROO^{\bullet} \xrightarrow{18} 2RO^{\bullet} + O_2$$

Scheme 3. Major reaction steps in iron porphyrin catalysed decomposition of peroxyacids and hydroperoxides.

radical (reaction 4) followed by superoxide addition to radical II.

Based on this knowledge, we can conclude that the oxidation of luminol by organic peroxy compounds should be strongly dependent on the type of oxidant. In this paper, we will describe results from our study of the CL reaction of luminol with peroxy compounds.

2. Experimental details

2.1. Instruments

In experiments described in this paper, we used the HPLC apparatus (shown in Fig. 1) as a flow injection system. It consists of a Waters 600 Controller used to deliver an eluate stream, a Waters 2487 Dual Absorbance Detector, a Waters 717 plus Autosampler, a Waters 474 Scanning Fluorescence Detector with the light source switched-off and two post-column reagent-delivery pumps (Waters Reagent Manager and PYE Unicam LC-XPD pump). Connections were made by a 0.009″ tube. Peroxides were separated on the Waters Nova-Pak Phenyl 3.9 mm × 150 mm Cartridge RP Column. The temperature of each stream and column was ambient (25 ± 4 °C) but each set

Table 1
Summary of rates of reactions involved in luminol oxidation

Reaction	Rate constant	Reference	Porphyrin/oxidant
1	$8.7 \times 10^9\ M^{-1}\ s^{-1}$	[15]	
2	$2.3 \pm 0.3 \times 10^8\ M^{-1}\ s^{-1}$	[7]	
3	$5.0 \pm 0.5 \times 10^8\ M^{-1}\ s^{-1}$	[7]	
4	$5.5 \pm 1 \times 10^2\ M^{-1}\ s^{-1}$	[7]	
5	$5 \pm 2 \times 10^7\ M^{-1}\ s^{-1}$	[7]	
6	$4 \pm 1 \times 10^6\ M^{-1}\ s^{-1}$	[7]	
7	$2.2 \times 10^5\ s^{-1}$	[6]	
10	$<10^{-6}$ s (lifetime)	[5]	
11	6.1 ± 0.2 ns (lifetime)	[16]	
12	$12 \pm 3\ M^{-1}\ s^{-1}$	[14]	Protohemin mono-3-(1-imidazoyl) propylamide monomethyl ester
13	$2.3 \times 10^6\ M^{-1}\ s^{-1}$	[17]	Horseradish peroxidase
14	$7.2 \times 10^4\ M^{-1}\ s^{-1}$	[17]	Horseradish peroxidase
15	$4\ M^{-1}\ s^{-1}$	[14]	Protohemin mono-3-(1-imidazoyl) propylamide monomethyl ester/*t*-BuOOH
16	$10^7\ M^{-1}\ s^{-1}$	[18]	Iron(III) tetrakis (pentafluorophenyl) porphyrin chloride/*t*-BuOO$^\bullet$
17	$1 \times 10^5\ M^{-1}\ s^{-1}$	[14]	Hemin/3-chloroperoxybenzoic acid
17	$7.6 \pm 0.2 \times 10^3\ M^{-1}\ s^{-1}$	[14]	Protohemin mono-3-(1-imidazoyl) propylamide monomethyl ester/3-chloroperoxybenzoic acid
18	$10^3\ M^{-1}\ s^{-1}$	[19]	*t*-BuOO$^\bullet$

of experiments measuring influence of flow rate, substitution, etc were carefully temperature controlled and not exceeding ±1 °C.

2.2. Reagents

Methanol HPLC grade was supplied by Merck, luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) 97% (Aldrich), hemin (chloro(protoporphyrinato) iron(III)) ≥98.0% (HPLC) (Fluka), NaOH and KOH puriss p.a. (POCh Gliwice). *tert*-Butyl hydroperoxide (1,1-dimethylethyl hydroxiperoxide) (reagent grade) from Merck. Cumyl hydroperoxide (1-methyl-1-phenylethyl hydroperoxide) technical grade (PKN Orlen) was purified by the precipitation of sodium salt [20]. Peroxyesters were synthesized from cumyl hydroperoxide and acyl chlorides, under PTC conditions [21], and purified by crystalisation. 4-Fluorobenzoyl chloride; 3-chlorobenzoyl chloride; 4-bromobenzoyl chloride; benzoyl chloride; 4-nitrobenzoyl chloride (Fluka); 3-nitrobenzoyl chloride; 3-methylbenzoyl chloride; 4-methylbenzoyl chloride; 4-chlorobenzoyl chloride; 4-methoxybenzoyl chloride (Merck); 4-*tert*-butylbenzoyl chloride; 4-cyanobenzoyl chloride (Aldrich) were reagent grade. Diacyl peroxides were obtained from acyl chlorides and hydrogen peroxide [22], and purified by crystalisation. Peroxy acids were synthesized according to [23] and used without further purifications. Hydroperoxides were prepared from corresponding alcohols through methanosulfonates [24,25]. Peroxide concentration in all products was calculated using active oxygen content, determined by iodometric titration [26,27]. Solutions of peroxy acids, peroxyesters and diacyl peroxides were prepared in $CHCl_3$, hydroperoxide solutions were prepared in methanol. Chemiluminescence reagents were dissolved in HPLC grade water. HPLC solvents were degassed by helium 25 ml min^{-1}, solutions of post-column reagents (stable for a few days) by vacuum.

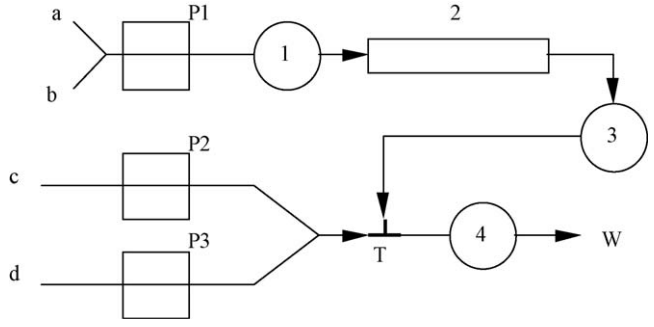


Fig. 1. Schematic diagram of the flow system for the measurements of CL intensity: (a) and (b) methanol, water; (c) and (d) post-column reagents solutions; (P1)–(P3) pumps; (1) autosampler; (2) HPLC column; (3) UV detector; (4) CL detector; (T) T-connector; (W) waste.

2.3. Chemiluminescence reaction

The typical reaction system applied in many CL technique consists of luminol, H_2O_2, base, and catalyst. In this study we used NaOH or KOH as a base and hemin as a catalyst. Hydrogen peroxide was replaced by organic peroxy compounds and we investigated how the CL signal was affected by structure of the oxidant.

Some peroxy compounds are relatively unstable and also difficult to obtain and store in pure form. The presence of other substances in a peroxide sample may strongly affect the CL signal. To avoid contamination of peroxyacids, diacyl peroxides and hydroperoxides with hydrogen peroxide, and peroxyesters with hydroperoxide we purified our reagents until only one peroxy compound was present per sample when measuring active oxygen content.

Additionally HPLC column allow simultaneous separation the peroxy compounds from any traces impurities during CL measurements. UV detection allowed careful control of separation process.

Column eluate (containing a sample of peroxides with a mobile phase of methanol (70–90%) and water (30–10%)) after leaving UV detector was merged with reagents stream delivered by pumps (P2) and (P3) (Fig. 1) via T-connector. After entering the flow cell of CL-detector (4) the emitted light was recorded.

The sensitivity setting (gain) was different for each substance and varied from 1 to 1000. The emission wavelength was set to 440 nm; the slit was set to 40 nm, and a digital filter of 3 s response time was chosen. Reaction time was altered by the flow rate of all streams, whereas the concentration of reagents was altered by changing the proportion of solutions delivered by pump (P2) and (P3). The flow intensity of eluate was equal to the sum of the flow of reagents delivered by pump (P2) and (P3). Pump (P3) was used only during the study of measurement conditions. The volume of T-connector was 18 μl (calculated from the weight of liquid that filled it) and the tube between the connector and the detector had a volume of 1 μl (calculated from its length). The total flow intensity varied from 0.6 to 3 ml min^{-1}, so the time between the beginning of the reaction and when it entered the detector ranged from 0.4 to 1.9 s. In that range, CL versus time profiles were measured for peroxybeznoic acid, dibenzoil peroxide, cumyl peoroxybenzoate and cumyl hydroperoxide.

2.4. Selecting post-column reagents concentration

For each experiment, two solutions of different concentrations of the investigated reagent were prepared. Pump (P2) and (P3) delivered them in proportion varying from 0 to 100%. The total flow was equal to the flow rate from pump (P1). The concentration of base, hemin and luminol (in that order) was tested for four model compounds: peroxybenzoic acid, cumyl peroxybenzoate, dibenzoyl peroxide and cumyl hydroperoxide. The initial concentration of luminol was 0.1 g dm^{-3} and for hemin, 0.1 g dm^{-3}, and was changed to the optimum concentration in the next experiments.

2.5. Emission spectrum

The emission spectrum was measured during the reaction of cumyl hydroperoxide by changing the emission wavelength setting of the CL detector. Three points for each wavelength were registered. Concentrations of post-column regents were given in 3.1. The flow rate was 1 ml min^{-1}. In the range of 435–445 nm, the highest signal was registered and a value of 440 nm was used in all subsequent experiments. The spectrum is shown in Fig. 2.

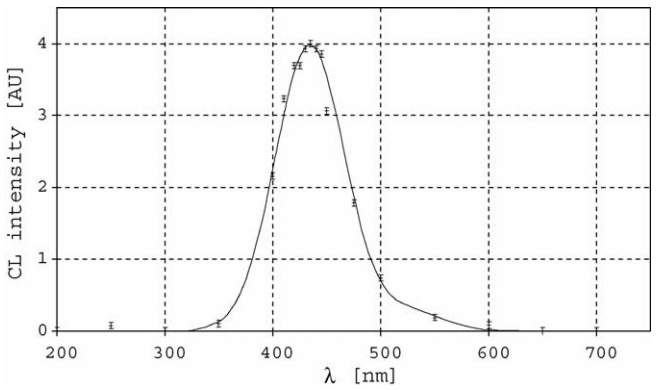


Fig. 2. Emission spectrum for the reaction of cumyl hydroperoxide with luminol.

2.6. Alcoholysis of diacyl peroxides and peroxyesters

Solutions of peroxides were prepared in $CHCl_3$, whereas solutions of NaOH were prepared in methanol. In the case of peroxyesters, the concentration of NaOH and peroxyester was 0.008 mol dm^{-3}. Diacyl peroxides react more rapidly with methanol so the concentration of NaOH was 0.0013 mol dm^{-3} and the concentration of peroxide was 0.0014 mol dm^{-3}. Solutions were mixed in the proportion of 1:1 and a sample of the reaction mixture was analysed by HPLC. The reaction of diacyl peroxides was stopped at a certain time by the addition of 30 μl of 0.0028 mol dm^{-3} HCOOH to a 500 μl sample of the reaction mixture. The decay of peroxide was used to calculate the reaction rate between a peroxide and methanol.

3. Results

3.1. Influence of post-column reagent concentration on CL intensity

The influence of concentrations of reagents on the CL reaction were studied in order to obtain maximum CL intensity for each group of peroxides and the best conditions for measurements of light quantity in relation to the amount of peroxide.

3.1.1. Effect of sodium and potassium hydroxide concentration on CL intensity

In the range of 0.005–0.8 mol dm^{-3}, the effect of the concentration of KOH and NaOH in post-column-reagent solution on the CL reaction was tested. The influence of base on CL intensity is shown in Fig. 3. Maximum performance was achieved when the concentration of NaOH in post-column reagents stream was 0.1 mol dm^{-3} (in the case of dibenzoyl peroxide and peroxybenzoic acid), 0.2 mol dm^{-3} (for cumyl peroxybenzoate) and 0.04 mol dm^{-3} (for cumyl hydroperoxide). However, 0.05 mol dm^{-3} solution was used instead, because of better solubility of luminol and hemin in more basic media. Similar results were obtained when KOH was used and we chose NaOH as a base in subsequent experiments. Dialkyl peroxides gave no CL signal and were excluded from investigations.

3.1.2. Effect of hemin concentration on CL intensity

The effect of the hemin concentration in the post-column reagents stream on CL intensity was studied in the range from 0 to 0.25 g dm^{-3}. Luminol concentration was 0.1 g dm^{-3} and NaOH concentration was suitable for each tested compound and was given in Section 3.1.1. Results are shown in Fig. 4. The best results were obtained when concentrations of hemin were as follows: 0.009 g dm^{-3} in the case of dibenzoyl peroxide and peroxybenzoic acid, 0.08 g dm^{-3} for cumyl peroxybenzoate, 0.1 g dm^{-3} for cumyl hydroperoxide.

3.1.3. Effect of luminol concentration on CL intensity

The effect of the concentration of luminol in solutions was evaluated from 0 to 0.4 g dm^{-3}. The concentration of hemin and NaOH was suitable for each peroxide and is given above. It is clear from Fig. 5 that increasing the concentration of luminol increases the CL signal, but the effect reaches a plateau when the

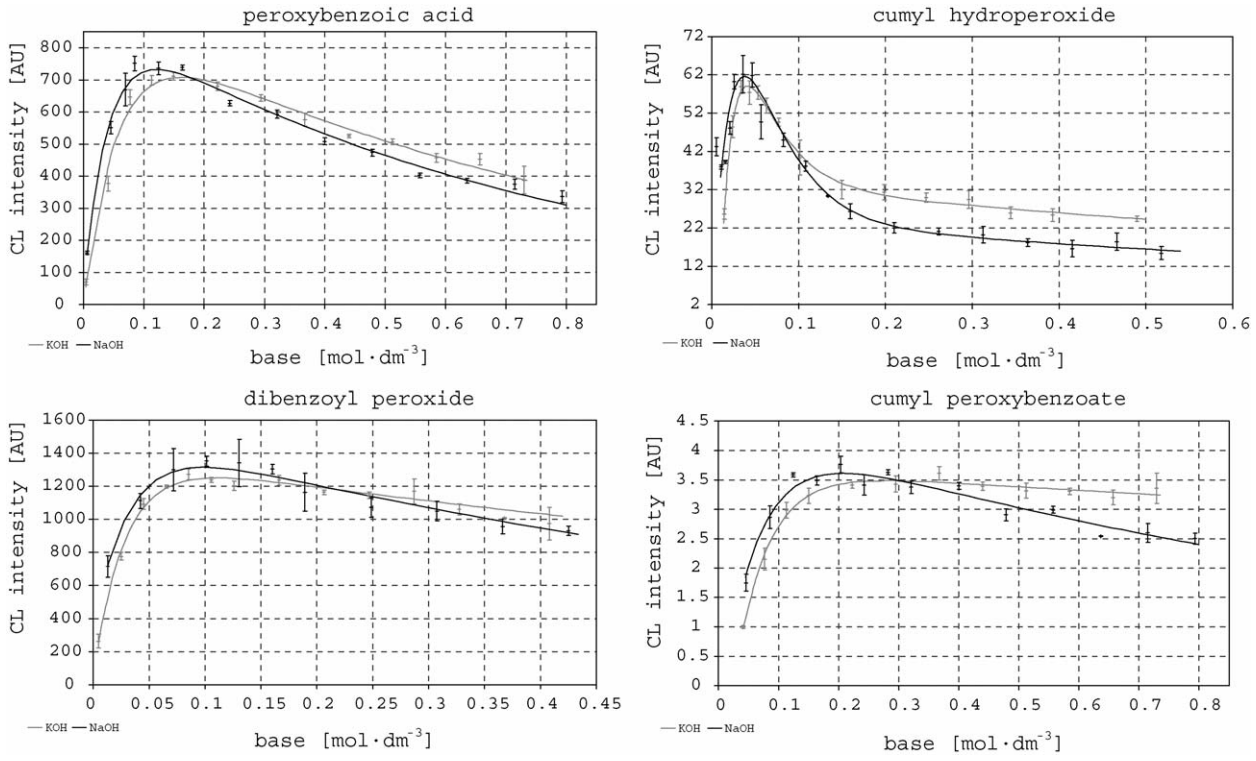


Fig. 3. Influence of KOH and NaOH concentration in post-column reagents stream on the CL signal obtained in the reaction of peroxybenzoic acid, cumyl peroxybenzoate, dibenzoyl peroxide, and cumyl hydroperoxide. Concentration of luminol was 0.1 g dm^{-3} and concentration of hemin was 0.1 g dm^{-3}.

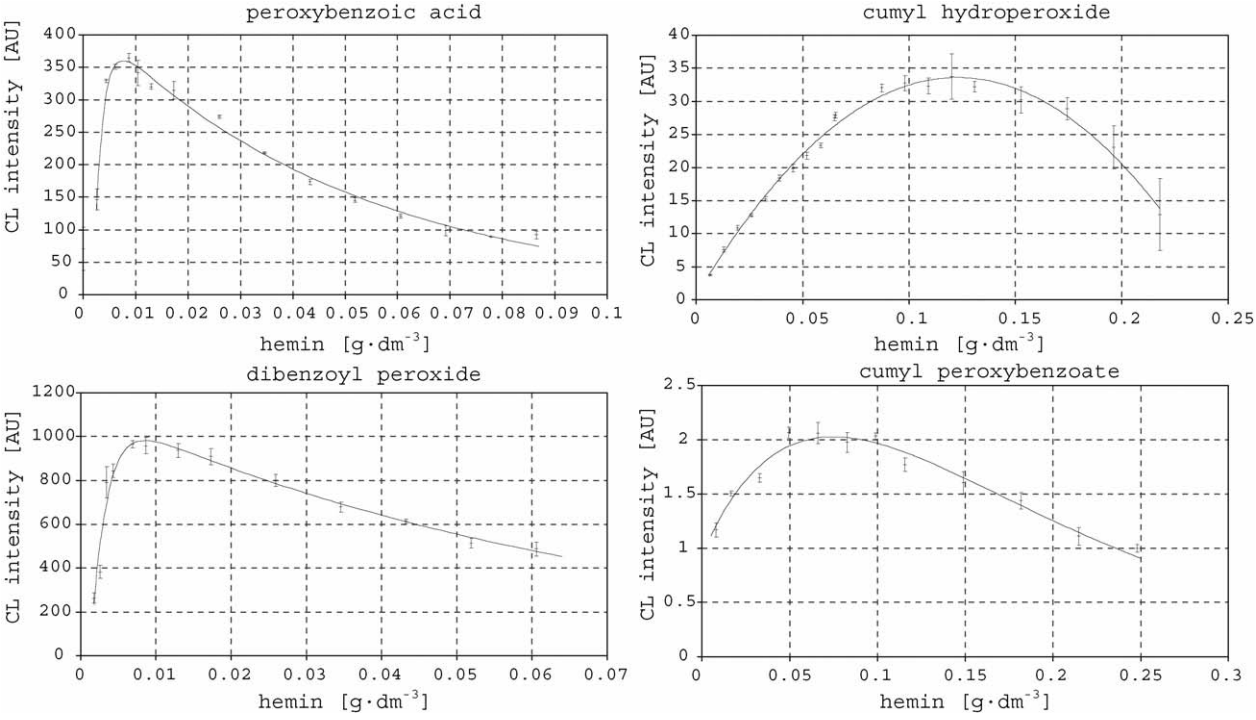


Fig. 4. Influence of the hemin concentration in the post-column reagents stream on the CL signal obtained in the reaction of peroxybenzoic acid, cumyl peroxybenzoate, dibenzoyl peroxide and cumyl hydroperoxide. Concentration of luminol was 0.1 g dm^{-3}.

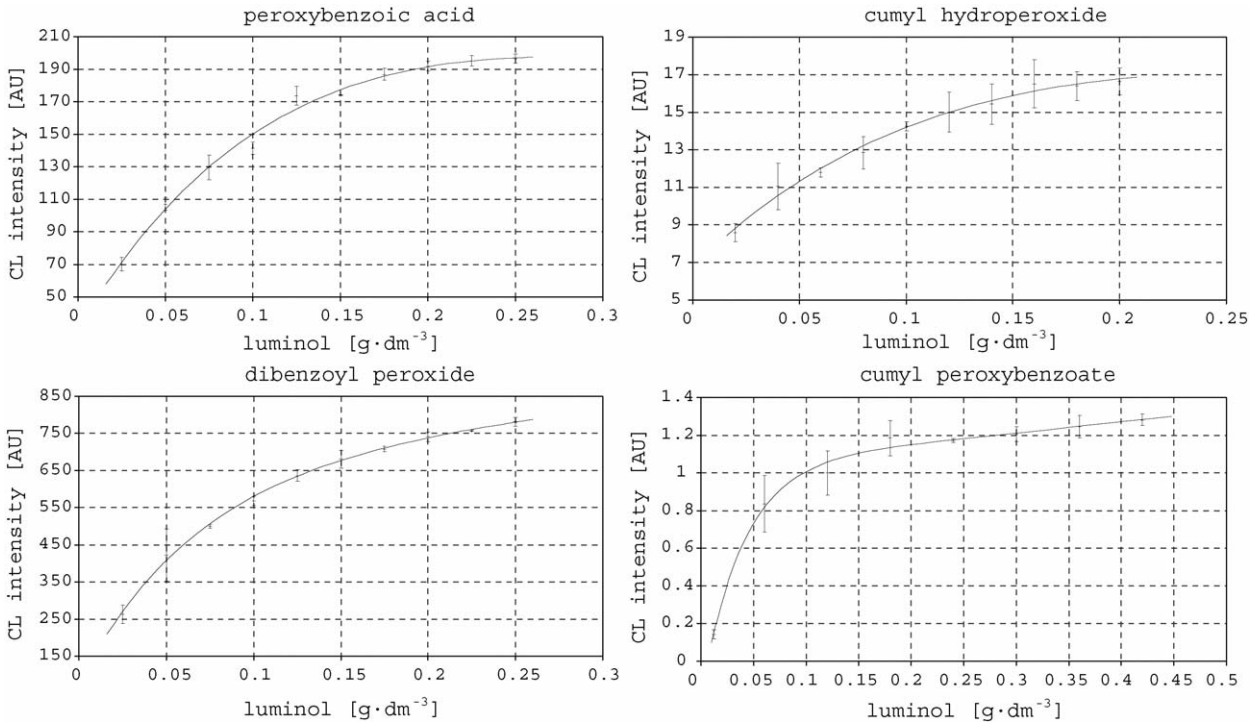


Fig. 5. Influence of the luminol concentration in post-column reagents stream on the CL signal obtained in the reaction of peroxybenzoic acid, cumyl peroxybenzoate, dibenzoyl peroxide and cumyl hydroperoxide.

amount of luminol is higher than 0.2 g dm^{-3}. We used solutions of 0.15 g dm^{-3} of luminol in subsequent measurements of all peroxy compounds.

3.2. CL versus reaction time

The effect of flow rate on the intensity of CL generated by organic peroxides was studied in the range from 0.4 to 1.5 ml min^{-1}. We did not use higher flow rates because they led to high backpressure at the HPLC column. The dynamic profiles of the CL reactions of four model peroxides were obtained using suitable solutions of post-column reagents for each substance. In the case of dibenzoyl peroxide and peroxybenzoic acid, we were able to register only the decay of the CL signal. Cumyl hydroperoxide and cumyl peroxybenzoate appear to react less rapidly with luminol and we determined the flow rate of CL to reach its maximum. Results are shown in Fig. 6. Flow rates that were used in subsequent experiments are as follows: peroxy acids 1.1 ml min^{-1} diacyl peroxides 1 ml min^{-1}; hydroperoxides 1.0 ml min^{-1}; peroxyesters 1.2 (higher intensity) and 0.73 ml min^{-1} (lower intensity). In the case of peroxy acids and diacyl peroxides, it is possible to obtain a higher CL signal by increasing flow rates above 1.1 ml min^{-1}; however the intensity of light generated by those compounds under the conditions we suggested was good enough.

3.3. Effect of substitutions on reactivity of peroxides in the reaction with luminol

Light emission accompanying luminol oxidation is a multistep process. As shown in Schemes 1–3, molecules of oxidant have two functions. First is the production of luminol with participation of the catalyst. Secondly is formation of endoperoxide VII through a nucleophilic attack (reactions 2, 5 and 8). When organic peroxide is the oxidant, luminol oxidation is not possible without breaking the C–OO bond. Substituents should affect the rate of such reaction and they would also affect the light yield if this reaction was rate limiting step.

In order to evaluate the effect of substitutions on reactivity of peroxides in the chemiluminescent reaction with luminol, we measured the amount of light generated by each substance in relation to its amount during the fixed step of the reaction determined in Section 3.2.

The investigated range was 1×10^{-8} mol to 1×10^{-7} mol for proxy esters, 1×10^{-8} mol to 1×10^{-7} mol for hydroperoxides, 1×10^{-8} mol to 8×10^{-8} mol for preoxyacids and 1×10^{-9} mol to 1×10^{-8} mol for diacyl peroxides. Separation conditions were identical for all groups of peroxides. Peak shape and retention time did not vary substantially among investigated compounds and we used the relation between peak area and amount of peroxide as a way of measure the light yield. In all cases the relation was linear and the logarithm of slope for the linear calibration of amount versus CL peak area was used to draw Hammet plots for all groups except hydroperoxides, where only relative intensity was shown in Fig. 7. In the case of peroxyesters, two plots were drawn for each flow rate (0.73 and 1.2 ml min^{-1}). Results are summarised in Figs. 7 and 8.

3.4. Effect of substitution on reactivity of diacyl peroxides and peroxyesters in alcoholysis

In order to determine the importance of the hydrolysis step of diacyl peroxides and peroxyesters in multistep CL oxidation

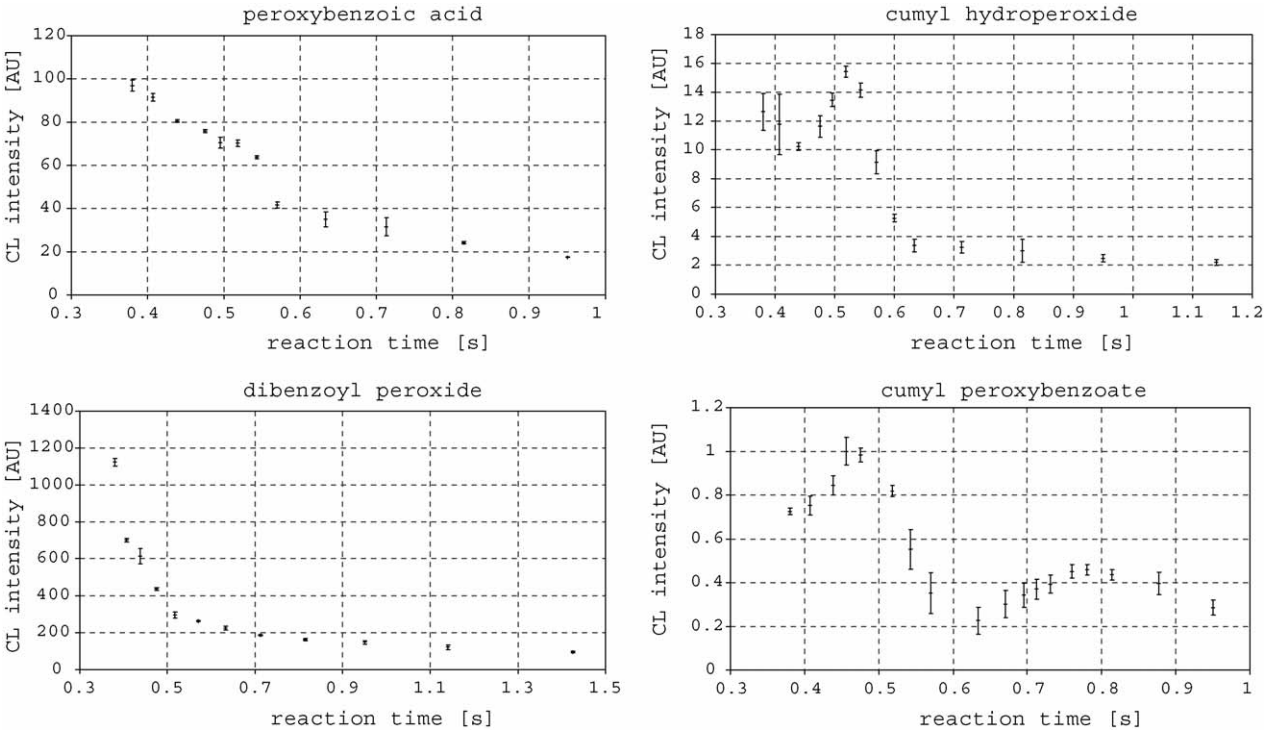


Fig. 6. CL time-profiles for luminol CL reactions with organic peroxides: peroxybenzoic acid, cumyl peroxybenzoate, dibenzoyl peroxide and cumyl hydroperoxide.

of luminol, we investigated the influence of substitution on the rate of base catalysed methanolysis of previously mentioned compounds. It is generally known that base catalysed solvolysis follows pseudo first-order kinetics, whereas diacyl peroxides reactions follow second-order kinetics [28]. Rate constants (monomolecular for peroxyesters (Table 2) and bimolecular for diacyl peroxides (Table 3)) were calculated on the basis of the decay of peroxy compounds during the reaction. Linear calibrations (Fig. 9) were obtained between substituent constants and the rate of reactions.

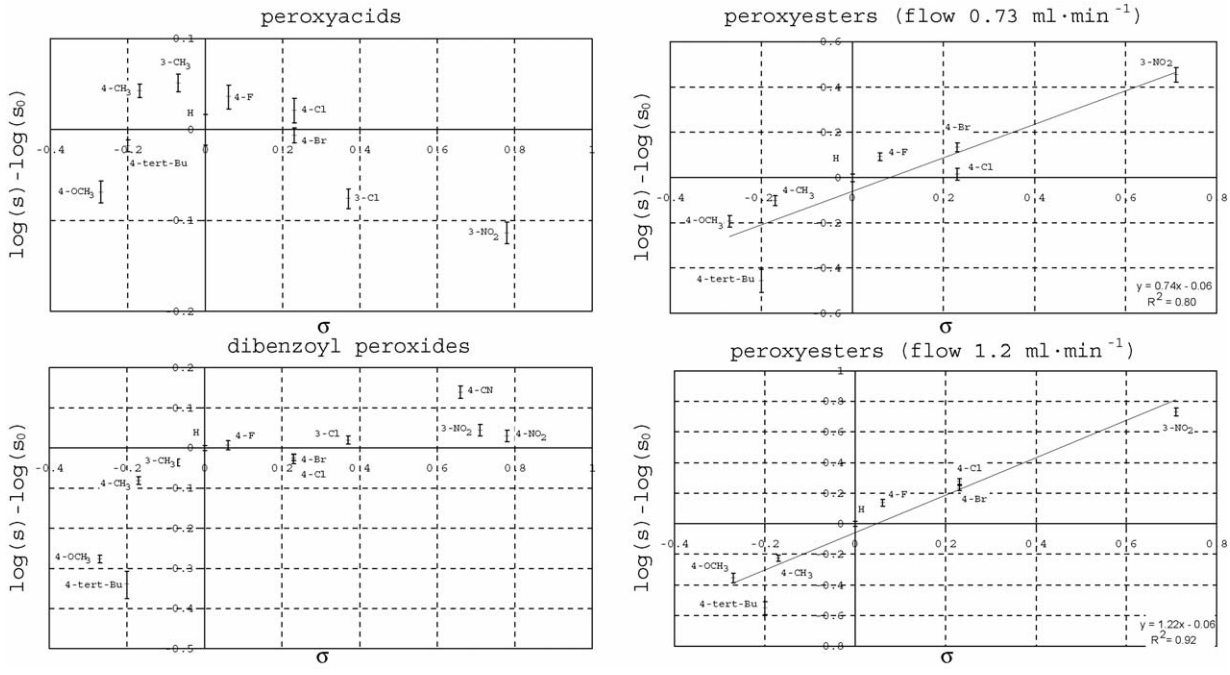


Fig. 7. Log of slope(s) of linear calibration between the CL peak area and the amount of peroxide vs. Hammet constants of substituents.

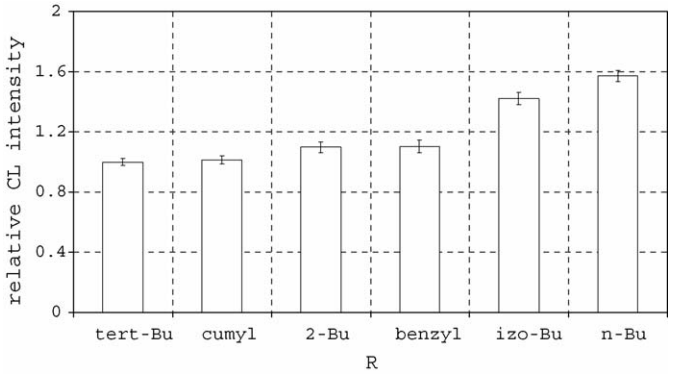


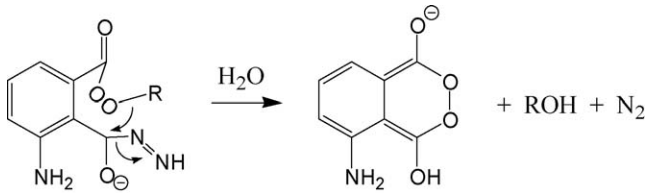
Fig. 8. Relative slope of linear calibration between the CL intensity in the luminol reaction and the amount of hydroperoxide (*tert*-Bu hydroperoxide slope = 1).

Table 2
Rate of methanolysis of substituted cumyl peroxybenzoates in methanol/$CHCl_3$ solution (50:50)

Substituent	Rate constant $\times 10^3$ (s^{-1})
H	73 ± 8
3-NO_2	586 ± 1
4-Br	189 ± 5
4-CH_3	28 ± 3
4-Cl	171 ± 2
4-F	123 ± 2
4-OCH_3	17 ± 1
4-*tert*-Bu	31 ± 5

Table 3
Rate of methanolysis of substituted dibenzoyl peroxides in methanol/$CHCl_3$ solution (50:50)

Substituent	Rate constant (M^{-1} s^{-1})
H	50 ± 5
3,3′-CH_3	52 ± 7
3,3′-Cl	$(36 \pm 2) \times 10^1$
3,3′-NO_2	$(12 \pm 2) \times 10^2$
4,4′-Br	261 ± 2
4,4′-CH_3	27 ± 2
4,4′-Cl	240 ± 4
4,4′-CN	$(10 \pm 2) \times 10^2$
4,4′-F	184 ± 5
4,4′-NO_2	$(8 \pm 2) \times 10^1$
4,4′-OCH_3	14 ± 3
4,4′-*tert*-Bu	27 ± 2



Scheme 4. Probable formation of endoperoxide in the hydroperoxide reaction.

4. Discussion

The rate of chemiluminescent oxidation of luminol differs for each group of peroxy compounds. Peroxyesters and hydroperoxides react slowly and maximum emission curves can be registered, whereas the reaction of peroxy acids and diacyl peroxides is fast and only the decay part of the emission curves can be obtained. For every investigated compound, different concentrations of base were allowed, in order to obtain the highest CL signal. The behaviour of peroxy acids and diacyl peroxides is similar. The reaction of hydroperoxides requires less basic conditions, whereas the reaction of peroxyesters needs five times higher concentration of NaOH than hydroperoxides to emit a satisfactory (but approximately 10 times lower) light yield. The fact indicates that peroxyesters are relatively inert toward luminol and hemin, and formation of hydroperoxide through hydrolysis is necessary to obtain light. On the basis of the methanolysis rate of peroxyesters (Table 2), we calculated the yield of hydroperoxide after 0.48 s in 0.1 M NaOH solution. The yield varied from 0.15% for *tert*-buthyl peroxybenzoate to 2.8% for 3-nitroperoxybenzoate and it is over 60% higher after 0.78 s. However, the light yield in the case of cumyl peroxybenzoate is only 10 times lower than for cumyl hydroperoxide. This can be explained by the fact that, in the presence of water under CL reaction conditions (a solution of 40% methanol and 60% water when rates of methanolysis were measured in 50% methanol and 50% of chloroform), the conversion is probably higher [29] than calculated values. Methanolysis is slow and its rate is strongly dependent on the substituents effect ($\rho = 1.5$ Fig. 8). There was a strong dependence on light yield in substitutions (Fig. 7) for peroxyesters—linear calibration for both Hammet plots for flow 1.2 and 0.73 (higher and lower maximum) gave $\rho = 1.2$ and 0.74, respectively, and also supported

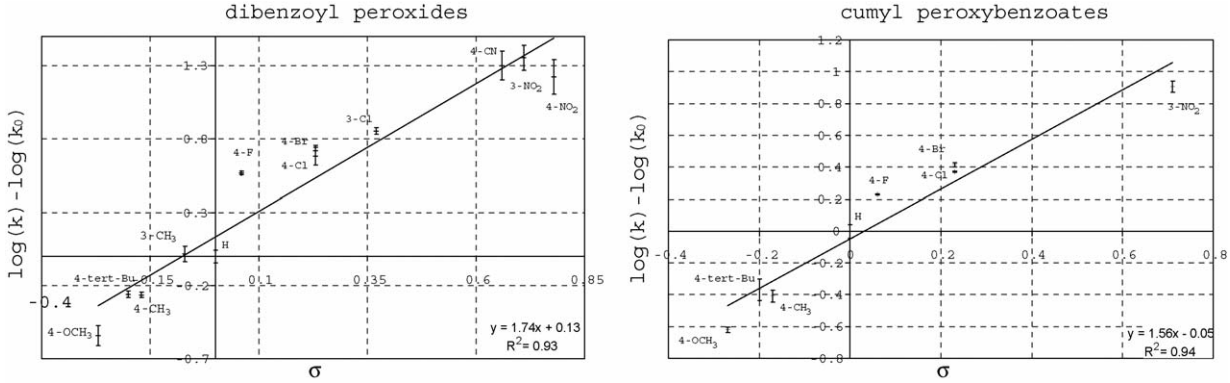
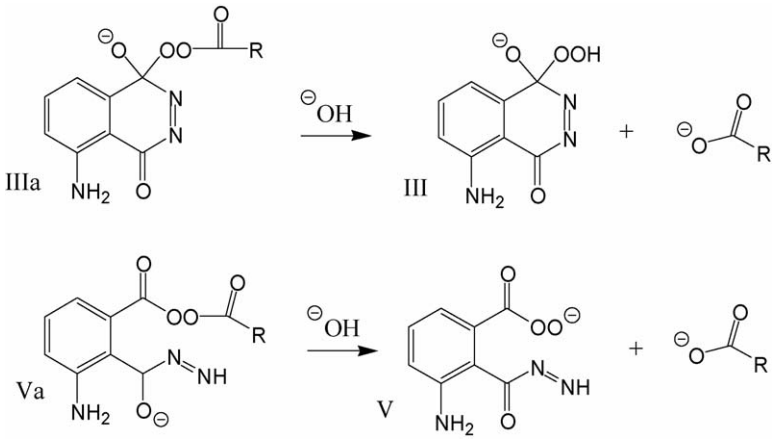


Fig. 9. The effect of substitution on alcoholysis rate of peroxyesters and diacyl peroxides.



Scheme 5. Possible intermediate in the peroxy acid reaction with luminol.

the assumption that hydroperoxide formation is crucial for light production.

Despite the fact that alcoholysis of diacyl peroxides is strongly dependent on substituent light ($\delta = 1.7$, Fig. 9), the amount is substantially affected only when substituent constants are negative. We used bimolecular rate constants of methanolysis to calculate the yield of peroxy acids after 0.57 s (resident time if flow rate = 1 ml min^{-1}) for five less reactive diacyl peroxides (with substituent: 4,4′-OCH_3; 4,4′-*tert*-buthyl; 4,4′-CH_3; 3,3′-CH_3 and dibenzoyl peroxide). Rate constants are as follows: 14, 27, 27, 36, 50 M^{-1} s^{-1} and conversions are 32%, 53%, 53%, 64%, 75%, respectively. If we take into account that the reaction of peroxy acids seems to be independent of substitution, it would be clear that the hydrolysis step is important only for compounds with the methyl, *tert*-buthyl and methoxy group. They react slower in the hydrolysis step and give a low yield of peroxy acid, affecting the amount of light.

The hydroperoxides reaction with luminol is slow and a small amount of light is obtained. We also registered that light yield decreases in order: primary > benzyl, secondary > tertiary hydroperoxides. Previous investigations [30] conclude that hydroperoxides react with hemin three times more slowly than hydrogen peroxide and approximately 1000 times more slowly than peroxy acids. This means that luminol radical formation is the slowest in hydroperoxide oxidation. Another intriguing problem is that C–O bond-breaking is necessary for the formation of endoperoxide VII. A reasonable explanation as to how the luminescent reaction proceeds is that the process is similar to the alkylation of hydroperoxide [31,32] and involves nitrogen expulsion as the driving force (Scheme 4).

All investigated peroxybenzoic acids are equally reactive despite the substitution, except 3-nitroperoxybenzoic acid which has a lower ability to generate light than the others—probably because of some kind of side reactions or its too rapid consumption in hemin oxidation. The most probable reaction course of peroxy acids and diacyl peroxides is consistent with our observations and should be as follows: the peroxy acid step (formed in hydrolysis when diacyl peroxide is a sample) reacts with diazaquinone to yield peroxyester IIIa. There are two further possible courses (Scheme 5): initial hydrolysis of peroxyester IIIa or diacyl peroxide Va to yield III or V (Scheme 5). The second possibility seems to be more probable, because this step should have a higher rate than peroxyester IIIa hydrolysis.

The rate of hemin oxidation by peroxy acids is dependent on substituents [19]. Similar reactions IIIa to V or Va should also be influenced by electron effects. However we did not register a substantial increase in light yield with the electron-withdrawing ability of substituents. This could be explained when one considers the following facts: oxidation of hemin by peroxy acids is very fast and is unlikely to be rate determining. Diacyl peroxides hydrolysis is also very fast (Table 3). Merenyi [6] stated that step 5 (Scheme 1) is an electron transfer and it is not sensitive to the substituent effect. The C–N bond cleavage rate in hydroperoxide V and luminol oxidation by oxene are independent of the oxidant structure. Alternatively, it is possible that peroxy acids react in a similar to hydroperoxides through the concerted decomposition of the C–N and O=C–O bond.

As a general rule we can conclude that the light yield in luminol oxidation is dependent on rate of the reaction between catalyst and peroxy compound and the rate of break C–O bond in either the parent or the intermediate peroxy compound.

With diacyl peroxides and peroxyacids, both reactions are very fast with strong light emission. Hydroperoxides reacts slower with iron porphyrins, as carbon–oxygen bond is of course stronger. These factors determine the lower light yield. Lower peroxyesters reactivity is due to slow formation of hydroperoxides via hydrolysis, even in strong basic media.

We found that highly reactive substances could be selectively analysed in the presence of other peroxy compounds. The lesser influence of structure on the hydroperoxides activity leads us to conclude that luminol-based methods are suitable for detection of various hydroperoxides also without use of expensive enzymes. This makes possible to apply luminol chemiluminescence for investigation of many industrial and natural oxidation processes.

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References

[1] S. Robinson, R. Bevan, J. Lunec, H. Griffiths, FEBS Lett. 430 (1998) 297–300.
[2] H.-C. Yey, W.-Y. Linn, Anal. Bioanal. Chem. 372 (2002) 525–531.
[3] M. Makinen, V. Piironen, A. Hopia, J. Chrom. A 734 (1996) 221–229.
[4] S. Baj, A. Chrobok, M. Cieslik, T. Krawczyk, Anal. Bioanal. Chem. 375 (2003) 327–330.
[5] S. Ljunggren, G. Merenyi, J. Lind, J. Am. Chem. Soc. 105 (1983) 7662–7666.
[6] J. Lind, G. Merenyi, T.E. Eriksen, J. Am. Chem. Soc. 105 (1983) 7655–7661.
[7] G. Merenyi, J. Lind, T.E. Eriksen, J. Phys. Chem. 88 (1984) 2320–2323.
[8] T.G. Traylor, F. Xu, J. Am. Chem. Soc. 112 (1990) 178–186.
[9] E.L. Bastos, P. Romoff, C.R. Eckert, W.J. Baader, J. Agric. Food Chem. 21 (2003) 7481–7488.
[10] T.G. Traylor, W.-P. Fann, D. Bandyopadhyay, J. Am. Chem. Soc. 111 (1989) 8009–8010.
[11] T.G. Traylor, C. Kim, W.-P. Fann, C.L. Perrin, Terahedron 54 (1998) 7977–7986.
[12] J. Van Der Zee, D.P. Barr, R.P. Mason, Free Radic. Biol. Med. 20 (1996) 199–206.
[13] P.K. Wittig, P. Travascio, D. Sen, A.G. Mauk, Inorg. Chem. 40 (2001) 5017–5023.
[14] T.G. Traylor, W.A. Lee, D.V. Stynes, J. Am. Chem. Soc. 106 (1984) 755–764.
[15] J.H. Baxendale, J. Chem. Soc., Faraday Trans. 1 (69) (1973) 1665–1677.
[16] Y. Haas, E. Wuerzberg, J. Phys. Chem. 83 (1979) 2692–2696.
[17] M.J. Cormer, P.M. Prichard, J. Biol. Chem. 243 (1968) 4706–4714.
[18] K. Kamaraj, D. Bandyopadhayay, J. Am. Chem. Soc. 119 (1997) 8099–8100.
[19] O. Almarsoon, T.C. Bruice, J. Am. Chem. Soc. 117 (1995) 4533–4544.
[20] Z. Kulicki, Zesz. Nauk. Pol. Sl., Chem. 183 (1967) 61.
[21] S. Baj, A. Chrobok, Polish J. Chem. 73 (1999) 1185.
[22] D.H. Hey, E.W. Walker, J. Chem. Soc. (1948) 2213–2220.
[23] Y. Ogata, Y. Sawaki, Tetrahedron 23 (1967) 3327–3332.
[24] H.R. Williams, H.S. Mosher, J. Am. Chem. Soc. 76 (1954) 2984–2987.
[25] H.R. Williams, H.S. Mosher, J. Am. Chem. Soc. 76 (1954) 2987–2990.
[26] L.S. Silbert, D. Swern, Anal. Chem. 30 (1958) 385–387.
[27] C.D. Wagner, R.H. Smith, E.D. Peters, Anal. Chem. 19 (1947) 976–979.
[28] M.L. Bender, Chem. Rev. 60 (1960) 53–113.
[29] M.L. Bender, W.A. Glasson, J. Am. Chem. Soc. 81 (1959) 1590–1597.
[30] T.G. Traylor, W.A. Lee, D.V. Stynes, J. Am. Chem. Soc. 106 (1984) 755–764.
[31] A.J. Bloodworth, K.H. Chan, C.J. Cooksey, J. Org. Chem. 51 (1986) 2110.
[32] N.A. Porter, J.C. Mitchell, Tetrahedron Lett. 24 (1983) 543.