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The Effect of Cyclodextrins on the Luminol-Hydrogen Peroxide Chemiluminescence

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Abstract

The action of different molar ratios of α, β, γ-cyclodextrin upon the chemiluminescence of the luminol-H_2O_2 in alkaline buffer Tris-HCl, pH = 8.5 has been evidenced. It was found out that α, β, γ- cyclodextrin have an antioxidant capacity, probably due to the free radicals (that are generate in the system) encapsulation in the their cavity. This behaviour depends on α, β, γ-cyclodextrin molar ratio; α-cyclodextrin and γ-cyclodextrin protects more efficiently against free radicals than β-cyclodextrin. These findings could be very important regarding the oxidative stress process.

Introduction

Natural cyclodextrins (CDs) are cyclic compounds obtained by enzymatic transformation of starch. α, β, γ-cyclodextrin, the first terms of this homologous series of oligomers containing 6, 7 and respectively 8 glucose units, tied to each other by 1–4 α-glicosidic bridges, Figure 1. CDs and their derivatives can form complexes by enclosing a variety of molecules of biochemical interest, due to their hydrophobic cavity, with a toroidal shape [1–4]. The diameter of the cavity interior ranges between 4.7 and 5.3 Å for α-CD, 6–6.5 Å for β-CD, 9–10 Å for γ-CD and the depth is 7.9–8 Å [2–4]. CDs act as solubilization and stabilization systems and are used as enzymatic models, as catalysts [5, 6] and in supramolecular catalysis [7]. It can be noticed [8] that aminoacids and protein oxidation by free radicals is inhibited in the presence of cyclodextrins. The hydrophobic cavity of CDs implies the access of the reagents to a new reaction medium in which there is a reactivity change. CD can be catalyst and mediator of the chemical reaction [9–12]. Studies regarding CDs effect on the chemiluminescence enhancement of lucigenin [13], peroxioxalat [14], luminol analogs [15] and acridine [16] were presented. The effect of β-CD and of the bovin serum albumine on the chemiluminescence of indol derivatives in alkaline medium was recently studied [17].

Luminol (LH_2) (5-amino-2,3-dihydro-1,4-phthalazinedione) is the most studied chemiluminescent compound (in water) due to its analytical applications [18]. Luminol-hydrogen peroxide in alkaline medium generates chemiluminmescence (Figure 2) [19]. In our previous papers, the photophysical and photochemical properties of luminol in aqueous medium and in different organic solvents have been studied [20–24]. The influence on luminol chemiluminescence and fluorescence of different additives as: Co^{2+}, Mg^{2+} and fluorescein (in water) [20], potassium superoxide and 18-Crown-6-Ether (in DMSO) [21], KOH (in DMSO) [22], KI, KBr, thiourea and riboflavin (in water) [24] has been evidenced.

The aim of this paper is the investigation of α, β and γ-cyclodextrin influence on luminol-hydrogen peroxide chemiluminescence, in alkaline solution and to establish if the cyclodextrins are involved in oxidative destructions induced by free radicals produced in the luminol-hydrogen peroxide system.

Experimental

Substances

The system luminol (LH_2) – hydrogen peroxide (H_2O_2) at concentrations of $[LH_2] = 2.5\times10^{-5}$M and $[H_2O_2] =$ 30 mM in 0.2 M Tris-HCl buffer, pH = 8.5, was considered as reference system. LH_2 and H_2O_2 are from Merck and the Tris buffer from Sigma. α-cyclodextrin is from Aldrich and β and γ-cyclodextrin from Merck. The solutions were fresh, prepared in distilled water.

Methods and apparatus

The CL measurements were undertaken with a chemiluminescence measuring device TD 20/20 Turner

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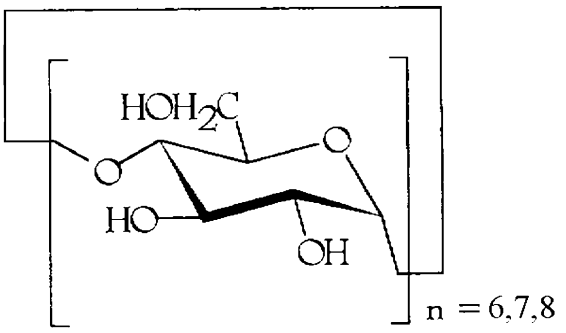

Figure 1. The chemical structure of α, *β*, γ-cyclodextrin (*n* = 6, 7 and 8, respectively).

Design, USA. The points on the plot were obtained by integrating the light signal on periods of 4 s. Five measurements were made and an average value calculated, obtaining a maximum 10% relative scattering of the results from the mean value. The work volume was 1000 μL solution.

The quenching of the CL emission (S %) was calculated according to the equation:

$$S(\%) = \frac{I_0 - I}{I_0} \times 100$$

where I_0 and I represent CL intensity measured for the reference system and for the reference system in the presence of α, *β*, and γ-cyclodextrin, respectively; both values were measured 5 s after the beginning of the reaction.

$I_{CL} = f(t)$ variation enable the determination of the rate constant of the reaction, for the upward part of the plot, $-k_2$, (attributed to the consumption of free radicals) as well as for the downward part of the curve, k_1, (attributed to the reaction of forming free radicals), considering that the CL reaction is of the first order. k_1 and k_2 values were determined using the following equation:

$$k = 1/\Delta t \times \ln(I_i/I_0),\ \Delta t = t_i - t_0$$

in which t_i represents the time at the i moment, t_0 represents the initial time, I_i is the intensity of CL signal at the moment i and I_0 is the intensity of the CL signal at the initial moment. The values of the rate constants were calculated on different time ranges, in this way: 5–100 s ($-k_2$) and 105–300 s (k_1), for α and γ-CD; 5–50 s ($-k_2$) and 55–300 s (k_1), for *β*-CD.

Resuts and discussion

The chemiluminescence technique is based on generating free oxygen radicals ($HO^{\bullet}$, $O_2^{\bullet-}$, $^1O_2$, $ROO^{\bullet}$) in a luminescence system and on studying the pro and anti-oxidant action of a molecule one is interested in [18, 19]. The luminol (LH_2)-hydrogen peroxide system in alkaline solution leads to aminophtalate dianion in an excited electronic state, compound that when de-excited emits a quantum light (a band with maximum at 430 nm). When introducing a new molecule of interest in this system, the CL signal will became higher or lower, fact attributed to an oxido-reducing process: when the CL signal increases, the molecule has pro-oxidating properties, while the decrease of the CL signal coresponds to reducing properties of the molecule.

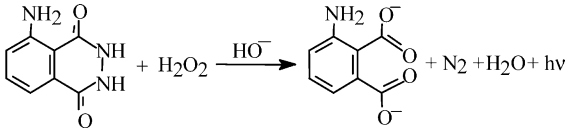

Figure 2. The luminol CL emission production [18, 19].

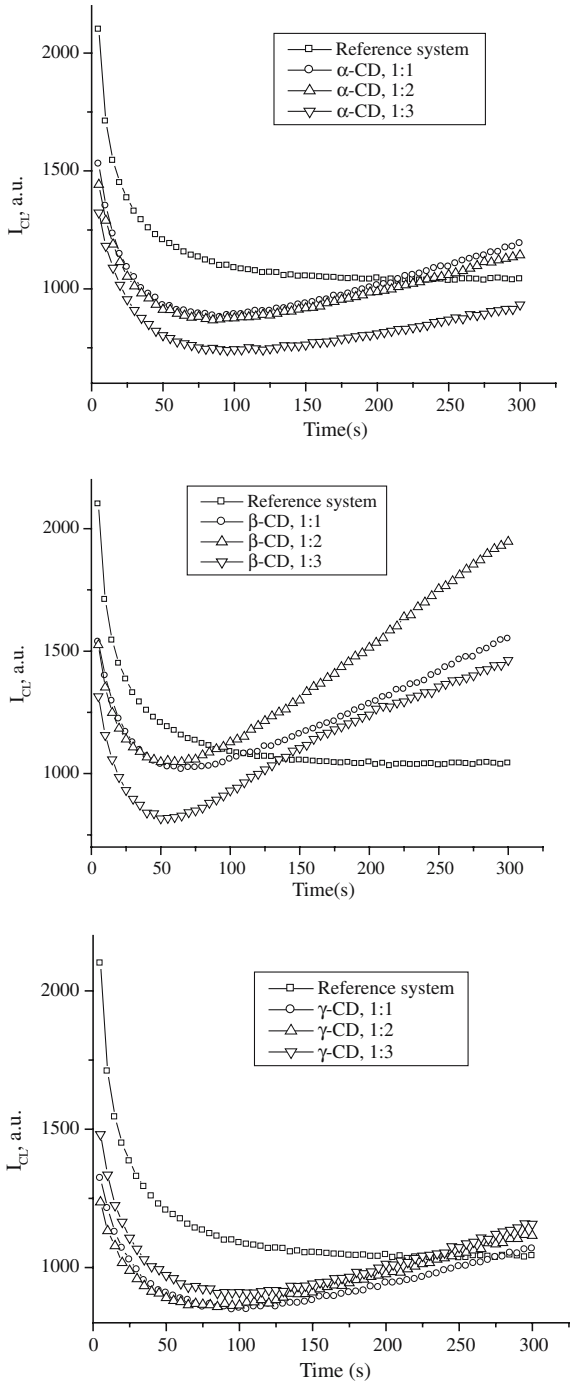

Figure 3. The CL emission variation of luminol-hydrogen peroxide system, in alkaline solution, Tris-HCl buffer pH 8.5, in the presence and in absence of α, *β* and γ-cyclodextrin; the molar ratio 1:1, 1:2; 1:3.

Figure 3 shows that α, β, γ-CD present a different behaviour in the system LH_2-hydrogen peroxide in alkaline solution, according to the molar ratio used in the process. As it can be noticed, α, β, γ-CD show an antioxidant effect signaled by a decrease of CL intensity with the reaction time. There are differences between α and γ-CD behaviour and β-CD behaviour: in the presence of α-CD or γ-CD, even after a long time, there is an inhibition of free radicals generation, while in the presence of β-CD free radicals are generated after a short time. We think that in a first phase, the process of trapping the free radicals produced by the system depends on the geometric characteristics of CD. β-CD cavity dimensions are between those of α and γ. From the Figure 3 one can notice that in the case of α and γ-CD the protective effect against oxidation is maintained for a longer time (200–250 s, respectively) than in the case of β-CD; this protective effect is anihilated after about 100 s. This can be explained if one considers that the small dimension of α-CD cavity does not favour the inclusion in it; the free radicals can get more easily into and out from the cavity of γ-CD.

We consider that β-CD can form more stable inclusion complexes and consequently the reactive species have access to a new reaction medium represented by β-CD cavity. So, there is a possibility to generate new radicals more quickly. Practically, there are two consecutive reactions: from the beginning of the reaction until 50 s later free radicals are consumed and included in the β-CD cavity (the rate constant of this reaction is in the range $0.66–0.81\times10^{-2}$ s^{-1}), after this period of time the CL intensity significantly increases ($k_1 = 0.15–0.21\times10^{-2}$ s^{-1}, Table 1). This can be due to a free radical chain propagation reaction that takes place in β-CD cavity. One can notice in Table 1 that the rate constants of the reactions that consume the free radicals are bigger than those of the reactions forming free radicals. The value of the CL emission quenching in the presence of α, β, γ-CD is situated in the range 27–41%, Table 1, in the case of γ-CD the effect being higher. The presence of CDs in the system has the tendency to trap partially the free radicals, depending on the reaction time and on the molar ratio of CD. This fact is important because one has tried to find different compounds of different types and from different sources with antioxidant properties.

Table 1. The kinetic parameters of CL process (efficiency of CL emission quenching and the rate constants) in the presence of different molar ratio of CDs (RS represent the CL generating system, luminol-H_2O_2 in alkaline solution, Tris- HCl buffer, pH 8.5)

System	S%	$k_1\times10^{+2}$, s^{-1}	$-k_2\times10^{+2}$, s^{-1}
RS	–		0.28
RS/ 1:1 α-CD	27	0.15	0.54
RS / 1:2 α-CD	31	0.14	0.50
RS/ 1:3 α-CD	37	0.11	0.57
RS/ 1:1 β-CD	27	0.15	0.81
RS/ 1:2 β-CD	27	0.21	0.66
RS/ 1:3 β-CD	37	0.18	0.77
RS/ 1:1 γ-CD	37	0.11	0.44
RS/ 1:2 γ-CD	41	0.12	0.35
RS/ 1:3 γ-CD	29	0.12	0.49

Conclusion

The effect of α, β, γ-cyclodextrin on the system that generates CL, luminol-hydrogen peroxide in alkaline solution, Tris-HCl buffer pH 8.5, was studied. From the obtained results the following conclusions were drawn:

- α, β, γ- cyclodextrin have a protective action against oxidative destructions caused by free radicals, this action depends on the molar ratio used; At 1:3 molar ratio of α, β, γ-cyclodextrin, the free radicals generation decrease.
- due to the β-cyclodextrin cavity dimension, this molecule could be a medium for free radical chain generation in time.

Therefore, cyclodextrins have a tendency to trap partially free radicals, this fact being dependent on the reaction time as well as on the cavity dimension and on the molar ratio of cyclodextrins.

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