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SULPHOACETIC ALDEHYDE – A NEW CHEMILUMINESCENCE ENHANCER

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It was shown that sulphoacetic aldehyde is the main product of the oxidative desamination of taurine monochloramine in the presence of H_2O_2. As it was confirmed by the HPLC method, in the alkaline solutions, taurine monochloramine deamination leads to the sulphoacetic aldehyde accumulation in the assay buffer. Our studies reveals that this product itself acts as a chemiluminescence (CL) enhancer in the both ABEI/taurine monochloramine/H_2O_2 and ABEI/H_2O_2 systems. Additionally it was shown that sulphoacetic aldehyde is an effective enhancer in the mM range of concentrations and its action is synergistic with *p*-iodophenol (PIP), a widely used phenolic enhancer. The proposed CL system improves the sensitivity of ABEI labeled antibodies (IgG) CL assays in microplate luminometers.

INTRODUCTION

The sensitivity of (iso)luminol based chemiluminescence (CL) assays is increased in the presence of *p*-iodophenol (PIP) (Thorpe & Kricka, 1986) or other phenolic derivatives as phenylylboronic acids (Kricka & Xiaoying, 1995) and some hydroxybenzylidene-cyclopentenediones (Hori, Fujii, Kubo, Pan, Sako, Tada & Matsubara, 1994). These compounds enhance CL of the horseradish peroxidase (HRP)-mediated oxidation of (iso)luminol in the presence of hydrogen peroxide. It was suggested that the presence of strong phenolic enhancers in peroxidase system may increase phenoxy radical production which in turn leads to the more efficient oxidation of the (iso)luminol ring and concomitant light production. It was also shown that the reaction of hydrogen peroxide with taurine monochloramine generates long-lasting CL of N-(4-aminobutyl)-N-ethylisoluminol (ABEI) which is enhanced in the presence of PIP in alkaline solution (Olszowski, Olszowska, Stelmaszyńska & Krawczyk, 1997). This allowed to use the nonenzymatic H_2O_2/taurine chloramine/PIP system in assays of ABEI – labeled IgG with the label detection limit of 0.1 pmol/well in the microplate CL assays (Olszowski, Olszowska, Stelmaszyńska & Krawczyk, 1999). In this paper we identified the product of the reaction of taurine chloramine with hydrogen peroxide and examined its influence on CL of the system.

MATERIALS AND METHODS

Acetonitrile, acetone, taurine, HCl, sodium thiosulphate and ferric chloride were obtained from Fluka Chemie AG (Switzerland); sodium dibasic and monobasic phosphate, N-(4-aminobutyl)-N-ethylisoluminol (ABEI), 4-iodophenol (PIP), sodium hypochlorite, formaldehyde, acetaldehyde, hydroxyethanal, α-ketocapronic acid, α-ketovaleric acid and hydrogen peroxide from Aldrich (Germany); catalase, N-methylbenzothiazolone hydrazone (MBTH), NaOH, anti-human fibrinogen (IgG) and dimethyl adipimidate from Sigma (USA). NaOCl solutions were prepared prior to use from 0.5 M stock solution and standardized iodometrically. H_2O_2 solutions were prepared from 30% solution prior to use and standardized manganometrically. The assay buffer contained 1:1 v/v 0.2 M phosphate buffer pH 7.4 and 2 M NaOH. All solutions were prepared using water obtained from EASY pure RF Compact Ultrapure Water System, Barnsted, USA (18 mΩ). 10 mM taurine monochloramine solutions in 0.2 M phosphate buffer, pH 7.4, was prepared from HOCl and the conjugate of ABEI with anti-human fibrinogen (ABEI-IgG) was syntetized as previously reported (Olszowski *at al.*, 1999). 10 mM standard sulphoacetic aldehyde solutions were obtained in the reaction of cysteic acid with HOCl in 0.05 M phosphate buffer, pH 5.3 within 24 h at room temperature. Stoichiometric conversion of cysteic acid was confirmed with MBTH method (Paz,

Blumenfeld, Rojkind, Henson, Furfine & Gallop, 1965).

Sulphoacetic aldehyde formation: samples of the 10 mM taurine monochloramine and 10 mM H_2O_2 in assay buffer were incubated 30 min at the room temperature. The reaction was stopped by HCl addition to the final pH 7.0. An excess of taurine monochloramine and H_2O_2 was decomposed with stoichiometrical amount of thiosulphate and catalase, respectively. Carbonylic compounds were assayed by MBHT method and the cyanine derivatives formed were separated by the HPLC method. Separations were performed with useing a KONTRON system on a Nucleosil C-18 (4.6 × 250 mm) column. Spectrophotometric assays were performed with a Hitachi U-2000 spectrophotometer (Japan). Chemiluminescence (CL) was measured using an Anthos Lucy 1 plate luminometer (Austria) and 96-well black FluoroNunc Modules. CL of each sample was recorded with 2 sec integration time, monitored for 15–60 min and presented as relative lights units (RLU). The CL trigger reaction was initiated prior to the assay by mixing ABEI or ABEI-labeled IgG with the assay buffer containing components of the system (H_2O_2, taurine chloramine, PIP or/and sulphoacetic aldehyde). In a series of ABEI-IgG assays labeled IgG was hydrolysed in the assay buffer for 30 min at 37°C before CL trigger reaction was initiated.

RESULTS AND DISCUSSION

Taurine chloramine undergoes slow deamination in the presence of H_2O_2 in acidic and neutral solutions. The process is rapidly accelerated in alkaline assay buffer. The final product of this oxidative deamination was identified by the HPLC method as sulphoacetic aldehyde cyanine derivative (Fig. 1). Its structure was also confirmed by ^{1}HNMR spectra of its 2,4-dinitrophenylhydrazone (data not shown). During the course of the reaction of taurine monochloramine with H_2O_2, sulphoacetic aldehyde is accumulated in the assay buffer and may influence the long-lasting (hours) CL of the system.

The following systems (Fig. 2) were examined to check the influence of sulphoacetic aldehyde on the overall CL. All CL generating systems contained ABEI and H_2O_2, and different combinations of other components: taurine monochloramine, PIP or sulphoacetic aldehyde. The presented data show that sulphoacetic aldehyde itself increases the CL of the ABEI/H_2O_2 system in the presence of PIP (C1, C2) or when PIP is omitted (D1, D2). The same enhancing effect could be observed when taurine monochloramine is present in the samples. It is interesting that other aldehydes (formaldehyde, acetaldehyde, hydroxyethanal, glutaric aldehyde), ketones (propanone) and ketoacids (α-keto capronic and valeric acids) do not influence the CL of any systems presented. The enhancing effect

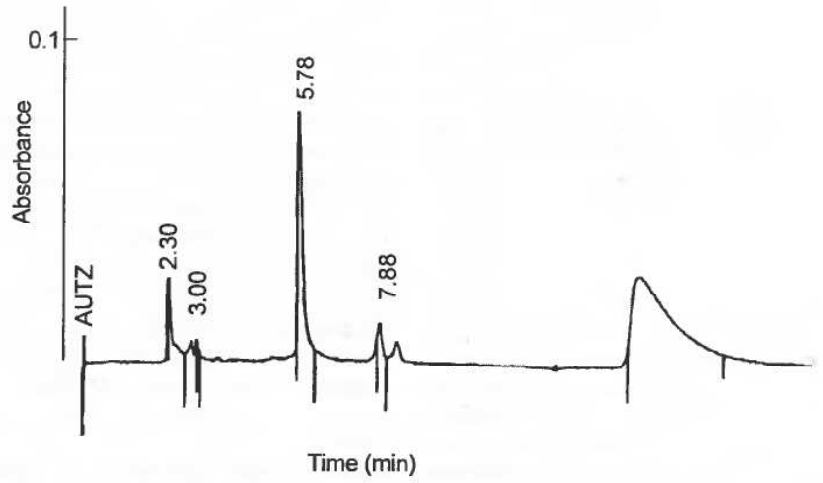

Fig. 1. HPLC identification of sulphoacetic aldehyde cyanine derivative. Aliquots of 20 µl of the MBTH derivative of the carbonyl compound formed in the reaction of taurine chloramine with H_2O_2 (30 min incubation time) were diluted 10 times with the eluent (1:1, v/v 5 mM phosphate buffer pH 7.0 and acetonitrile) and separated on Nucleosil C-18 column in isocratic conditions. Fractions were detected at 598 nm. Flow rate: 0.8 ml/min. Control samples containing standard solution of sulphoacetic aldehyde showed the same (5.78 ± 2 sec) retention time. Other peaks correspond to the unidentified compounds found in the blank sample without any MBHT derivative

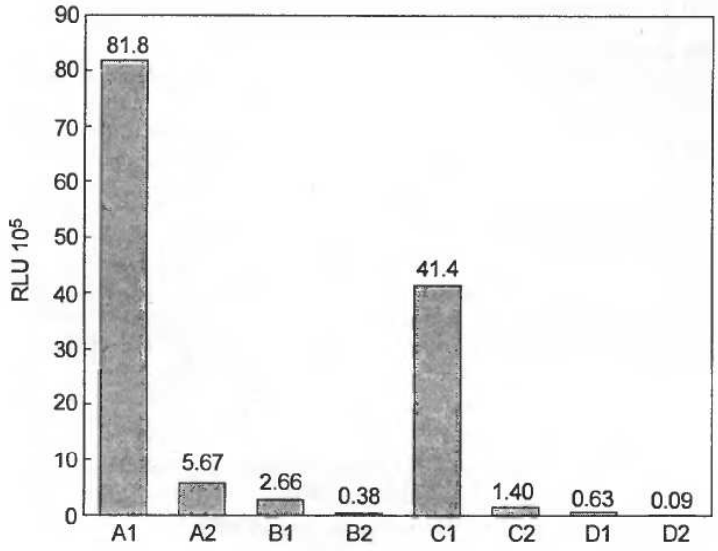

Fig. 2. The enhancing effect of sulphoacetic aldehyde on CL. Samples (280 µl) contained 1.2 mM PIP, 2.1 mM taurine chloramine and H_2O_2, 2.1 mM sulphoacetic aldehyde and 6.85 mM ABEI in the assay buffer (*). CI was recorded 30 min after ABEI addition. Samples in which H_2O_2 or ABEI were omitted showed CL comparable to background CL and did not exceed 4×10^3 RLU/30 min
(*):

Components Systems	Taurine Chloramine	H_2O_2	PIP	ABEI	Sulphoacetic aldehyde
A1	+	+	+	+	+
A2	+	+	+	+	–
B1	+	+	–	+	+
B2	+	+	–	+	–
C1	–	+	+	+	+
C2	–	+	+	+	–
D1	–	+	–	+	+
D2	–	+	–	+	–

is the greatest (up to 4000%) in the systems containing the both enhancers (PIP and sulphoacetic aldehyde). Since other aldehydes, ketones and ketoacids do not show any influence on the CL of ABEI/H_2O_2 and ABEI/H_2O_2/taurine monochloramine systems it could be concluded that the enhancing effect refers rather to the location of $-SO_3^-$ group in the close proximity of the carbonyl group than to the presence of carbonyl group itself. Although the mechanism of sulphoacetic aldehyde action is unknown, its enhancing effect could be attributed to enol formation in the strong alkaline solution as is suggested by changes of UV/VIS spectra (Olszowski *at al.*, 1999).

The sulphoacetic aldehyde-mediated enhancing effect depends on its concentration (Fig. 3) and seems to be essential for the mM range of concentrations. Since the production of the aldehyde via taurine chloramine deamination reaches the mM level within an hour (Fig. 1) it seems probable that at least a part of light emission is sulphoacetic aldehyde mediated.

Sulphoacetic aldehyde could act also as an enhancer in the systems containing ABEI-labeled proteins (IgG). In this type of CL methods the covalently bounded ABEI is released to the solution before the CL trigger reaction is initiated. Thus the assay buffer contains proteins which quench CL or may influence action of the enhancers. As it is shown in the Fig. 4 the sulphoacetic aldehyde still acts as an enhancer in such a system when used in mM concentration and improves the sensitivity of the ABEI labeled IgG assay up to 40 times for the ng/ml protein concentrations.

All the presented data suggest that H_2O_2/HO_2^- mediated oxidation of the isoluminol ring of ABEI is catalyzed by taurine monochloramine or by

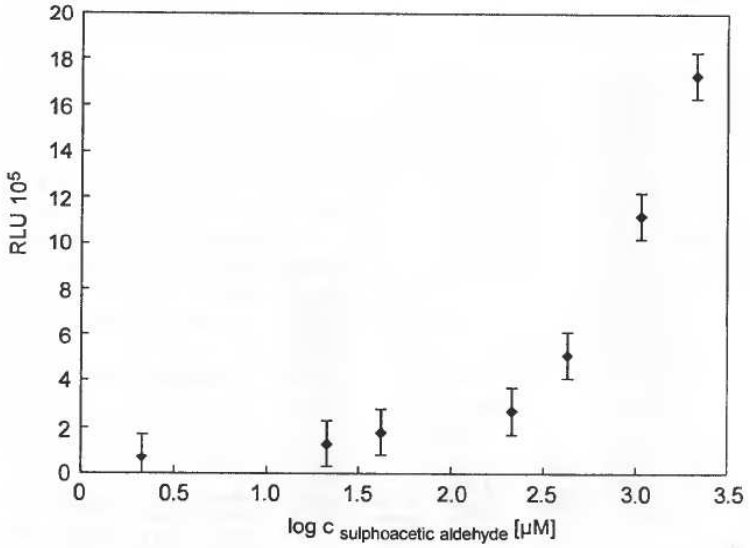

Fig. 3. IgG-ABEI chemiluminescence versus sulphoacetic aldehyde concentration. Samples contained various amounts of sulphoacetic aldehyde with 1.13 mM PIP, 4 μg/ml IgG-ABEI, 2.1 mM taurine chloramine and H_2O_2 in the assay buffer. The curve represents mean of four assays. The background CL did not exceed 4×10^3/30 min

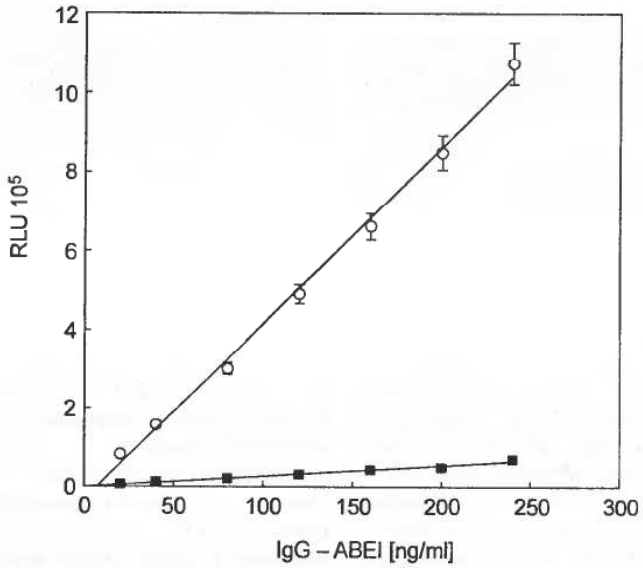

Fig. 4. Standard curves for the IgG-ABEI CL. Samples (280 μl) contained various concentrations of IgG-ABEI, 1.13 mM PIP, 2.1 mM taurine chloramine and H_2O_2 with (O) without (■) 2.1 mM sulphoacetic aldehyde in 2 M NaOH. Other conditions as in Fig. 3

product of its deamination, sulphoacetic aldehyde. Moreover, both enhancers (PIP and sulphacetic aldehyde) used together provide the best conditions for immunoassays by means of microtiter plates.

Acknowledgements

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