Luminescence 2006; 21: 31–35
Published online 15 August 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/bio.879 *ORIGINAL RESEARCH*

Chemiluminescence during rice seed imbibition at different temperatures

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Received 11 March 2005; revised 13 June 2005; accepted 14 June 2005

ABSTRACT: The chemiluminescence (CL) of rice (*Oryza sativa* L.) seeds at different temperatures and the CL spectra of rice seed, caryopses and seed coat were studied during early imbibition. Compared with the CL of barley (*Hordeum vulgare* L.) and soybean (*Glycine max* L. Merr.) seeds, the CL of rice seeds had a non-linear, logarithmic-like increase of intensity in the temperature range 30–50°C. The Van't Hoff coefficient $Q_{10} = I_{T+10}/I_T$ was equal to 2. The emission spectrum of whole rice seed, rice and coat had a greater proportion of red light during early imbibition, which led to the conclusion that the CL of rice seed during early imbibition arises partially from enzyme-catalysed reactions. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: rice seed; chemiluminescence; CL spectrum; Van't Hoff coefficient; imbibition

INTRODUCTION

Chemiluminescence (CL) is the generation of electromagnetic radiation as light through the release of energy from a chemical reaction. The study of CL of plants and its applications in agriculture has made significant progress. In recent years, evidence has accumulated to indicate that interactions between water and cereal significantly affect the processing, storage and quality of cereal products (1, 2). CL-based optical methods have recently been developed to monitor the deterioration of food. Previous studies have shown that the interaction between water and dry cereal grains, flour and bread products results in much stronger CL signals (3–6).

In general, seed germination can be separated into three distinct phases (7). Extensive work on seed vigour research utilizing materials from germination phases II and III has been reported. There are also a number of reports and reviews on seed ageing and the loss of viability attributed to the accumulation of free radicals (8). However, we were interested in germination phase I—early imbibition. We found that the seed vigour could be detected, based on the CL signals during early imbibition (6). Some reports showed that high-viability seeds were characterized by an accumulation of O_2 radicals in phase I of germination (9).

Slawinska and Slawinski have studied the influence of temperature on the CL of whole wheat grains treated with water in the temperature range 293–313°K and reported that the Van't Hoff coefficient Q_{10} was 1.44. They suggest that physical and not enzymatic processes ($Q_{10} = 2$) are critical for the generation of excited electronic states, which might lead to the observed CL (3). Our previous report (6) showed that rice seeds with a longer storage time have a lower intensity of CL during early imbibition. The germination rate of rice seeds correlates well with the intensity of CL. Therefore, we believe that the CL of rice seeds during early imbibition is related to the enzyme activities. In the present study, the CL of rice (*Oryza sativa* L.) seeds during early imbibition was investigated at different temperatures during early imbibition using Slawinski's method. The CL spectra of rice seed, caryopses and seed coat were studied during early imbibition, using a series of long pass filters.

MATERIALS AND METHODS

Materials

Rice seeds of *Oryza sativa* L. strain 8072-2 were obtained from Guangdong Academy of Agricultural Sciences. All the samples were taken in separate cloth bags, stored in a desiccator with silica gel and kept at

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Contract/grant sponsor: Chinese Natural Science Foundation of Guangdong Province, People's Republic of China; Contract/grant number: 031511.
Contract/grant sponsor: Science and Technology Project of Guangdong Province, People's Republic of China; Contract/grant number: 2002C20607.
Contract/grant sponsor: DAAD; Germany; Contract/grant number: WLC.

room temperature. Soybean (*Glycine max* L. Merr.) and barley (*Hordeum vulgare* L.) seeds were obtained from the International Institute of Biophysics Laboratory (IIB, Neuss, Germany). Healthy seeds were carefully selected for subsequent experiments.

Rice sample preparation

For measuring the changes of CL under different temperatures, samples of rice seed (0.60 g), soybean seed (0.808 g) and barley (1.30 g) were put into custom-built quartz cells ($2 \times 2 \times 3$ cm). In order to avoid photo-induced delayed luminescence, the quartz cell was kept in a self-regulating temperature-controlled chamber in the dark for 30 min prior to measuring the CL of the dry samples. After the average photon counts rate from the dry samples stabilized, distilled water (2 mL) at the same temperature was injected into the quartz cell through a light-tight autoinjector and the CL was measured immediately. The injection water was kept in the temperature-controlled chamber in the dark for 30 min prior to the injection to maintain the same temperature as the seeds.

Determination of CL under different temperature conditions

The CL radiation was detected with a photomultiplier (Thorn EMI type 9558 QA) cooled to –20°C to decrease the dark current. The photocathode (50 mm in diameter) was placed 15.5 cm away from the sample holder. The spectral sensitivity of the instrumentation was in the range 220–850 nm. Photon counts were stored in a 4096-channel scaler with a minimum dwell time of 2 μs. In our operational mode, each channel of the scaler records the number of pulses counted in a 1 s time interval. The measurement system had a completely automatic temperature-controlled light-tight sample chamber and the operations of actuation, checking and acquisition were managed by dedicated software though a PC. The data presented in this article have been obtained from the values accumulated in the first 1000 acquisition channels, corresponding to 1000 s. Before each experiment, samples and water were placed in a dark room for dark adaptation to avoid photo-induced delayed luminescence. The sample chamber (including samples and water) was regulated to the test temperature. All operations were performed in triplicate under a dim light (from the PC screen).

Determination of the CL spectrum

A series of long pass filters were used to measure the CL spectrum because the intensity of CL signals during rice seed imbibition cannot be measured with cut-off filters. These filters included WG320 (half-width nm > 320), GG375 (half-width nm > 375), GG495 (half-width nm > 495), RG610 (half-width nm > 610), RG665 (half-width nm > 665) and RG715 (half-width nm > 715). The CL spectra of rice seed (30 grains), caryopses (60 grains) and seed coat (peeled from 30 grains rice seed) from the early imbibition were measured as follows. First, CL was measured without any filters for 5 min and then measured again using filters WG320, GG375, GG495, RG610, RG665 and RG715 for 5 min, respectively. The time required for changing the filters was 2–3 s, therefore, before-and-after points (a total of five data points) were deleted due to filter changing. The mean CL intensity of WG320 was the ratio of the average value of five consecutive data points obtained with the filter WG320 to the average value of five consecutive data points obtained without filter (background CL). The mean CL intensity of GG375 was the ratio of the average value of five consecutive data points obtained with the filter WG320 to the average value of five consecutive data points obtained without filter. The CL intensity for 320–375 nm was the value of (WG320 – GG375) × background CL intensity. Similarly, the CL intensity for 375–495 nm was the value of (GG375 – GG495) × background CL intensity; the CL intensity of rest wavelengths can be deduced by analogy. All operations were performed three times at 30°C and 65% relative humidity in the dim light of a PC screen. The CL intensity data represent the average of three replicates.

RESULTS

Changes of CL of rice seeds at different temperatures during early imbibition

The influence of temperature on the CL of rice seeds, soybean seeds and barley seeds treated with water in the range 283–323°K is shown in Figs 1–3, respectively. Comparing the CL of soybean (Fig. 2) and barley (Fig. 3) seeds at different temperatures, the CL of rice (Fig. 1) seeds had higher intensity. The CL intensity increased with an elevation of temperature. Our data indicate that the water penetration (imbibition) results in a non-linear and logarithmic-like increase of CL intensity of the water-treated rice seeds.

According to the calculating method of the Van't Hoff coefficient ($Q_{10} = I_{T+10}/I_T$) by Slawinska *et al.* (3) in the range 303–323°K, the Van't Hoff coefficient Q_{10} in rice seeds is equal to 2. At values lower than 303°K, the Q_{10} decreases with the decrease of temperature. Between 303°K and 293°K, Q_{10} is equal to 1.71; between 283°K and 293°K, Q_{10} is equal to 1.12. Van't Hoff's rule suggests that the velocity of chemical reactions is increased two-fold or more for each temperature rise of 10°C. Our data suggest that enzymatic processes ($Q_{10} = 2$) (3) in the range 303–323°K are critical for the generation of

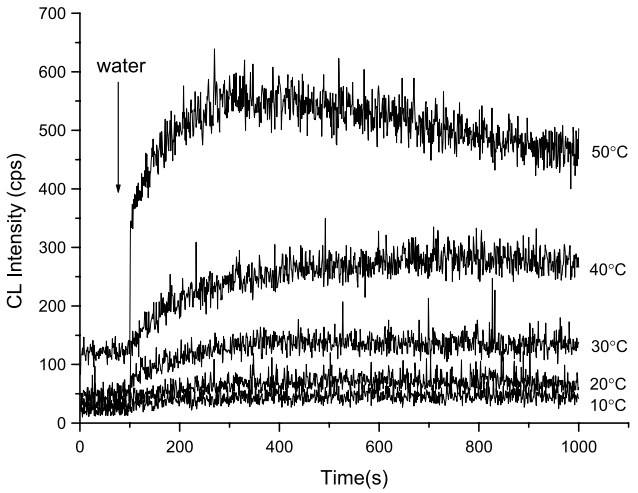

Figure 1. Kinetics curves of spontaneous CL from rice seed 8072-2 under different temperature conditions with distilled water (arrow).

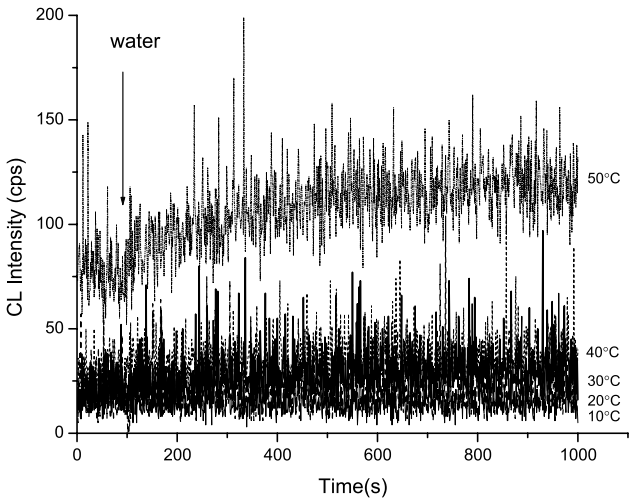

Figure 2. Kinetics curves of spontaneous CL from soybean under different temperature conditions with distilled water (arrow).

excited electronic states which lead to the observed CL. The temperature dependence of the CL reflects the rate-limiting step of processes generating molecules in excited electronic states:

$$M + H_2O^{Ki} \rightarrow M^{*Kf} \rightarrow M + h\nu$$

where K_i and K_f are the chemiexcitation and emission rate constants, respectively.

Because $Q_{10} = 2$ between 303°K and 323°K, the activation energy, E_a, of these processes can be evaluated from the data in Fig. 1. The E_a values, calculated from the simplified formula:

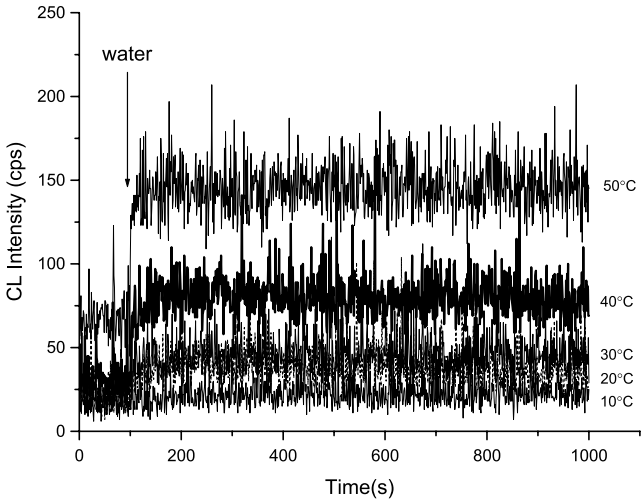

Figure 3. Kinetics curves of spontaneous CL from barley under different temperature conditions with distilled water (arrow).

$$\ln Q_{10} = 10E_a/10(T + 10)$$

where T = temperature, are 34.66 ± 4.1 kJ/mol at 313–323°K and 27.7 ± 5.3 kJ/mol at 303–313°K, respectively, assuming that $k_i >> k_f$ and I_{max} of CL is proportional to k_i at different temperatures, while T_1 and T_2 are in the range 283–323°K, $I_{max1} = I_0\exp(-E_a/RT_1)$ and $I_{max2} = I_0\exp(-E_a/RT_2)$, R = universal gas constant, value 8.314×10^{-3} kJ mol^{-1} K^{-1}. A simple transformation of these equations leads to the final form of the Arrhenius equation, $E_a = R \ln I_2/I_1 (T_1 - T_2)^{-1}$. Thus, the calculated value of E_a are 24.9 ± 3.6 kJ/mol at 303–323°K, 23.6 ± 4.0 kJ/mol at 293–303°K and 20.1 ± 1.9 kJ/mol at 283–293°K, respectively. Relatively low values of E_a suggest that the process of CL generation might be controlled by weak molecular interactions at 283–303°K, whereas the CL generation might be controlled by enzymatic interactions at 303–323°K.

The CL emission spectrum of whole rice seed, caryopses and seed coat during early imbibition

Long pass filters were used to measure the CL spectrum because the intensity of CL during rice seed imbibition cannot be measured with cut-off filters. CL spectrum of rice seed (30 grains), caryopses (60 grains) and seed coat (peeled from 30 grains rice seed) from the early imbibition were measured. The CL emission spectrum from rice seed, caryopses and rice seed coat during early imbibition are shown in Figs 4–6, respectively. The emission spectrum of intact rice seed, caryopses and seed coat had a greater proportion of red light during early imbibition.

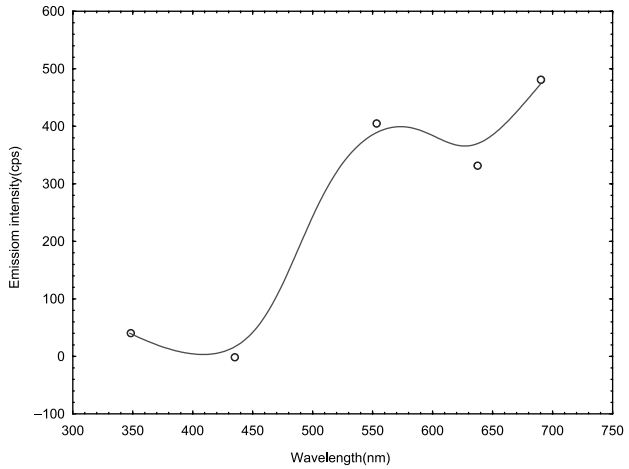

Figure 4. Emission spectrum from rice seed during early imbibition. Values represent the mean from three experiments.

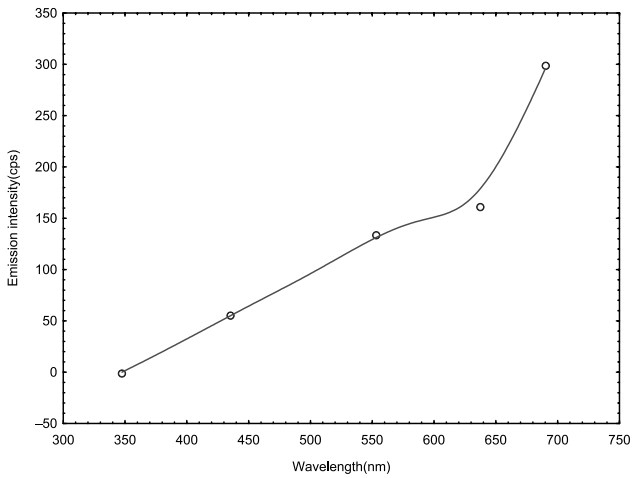

Figure 5. Emission spectrum from caryopses during early imbibition. Values represent the mean from three experiments.

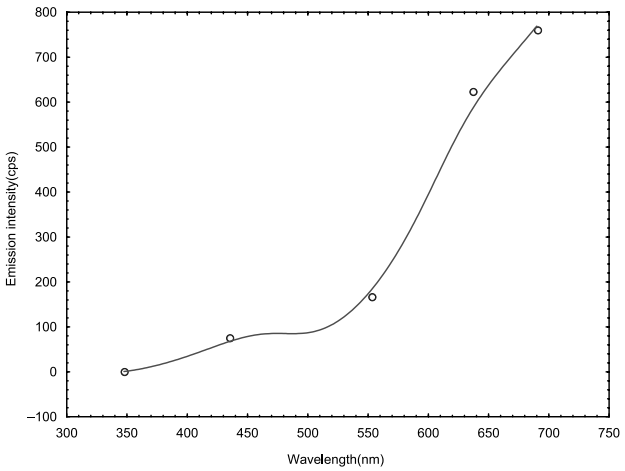

Figure 6. Emission spectrum from seed coat during early imbibition. Values represent the mean from three experiments.

DISCUSSION

CL is usually thought to be produced during the de-excitation (directly or indirectly) of high-energy excited carbonyl and singlet oxygen. The uptake of water by seeds is an essential and initial step toward germination. The water can rapidly enter the peripheral cells of the seed. Hence, metabolism can commence within minutes of imbibition (7). Deamination and transamination of amino acids begin in the first few minutes of imbibition (7). It has been reported previously that, during the initial steps of germination, isolated soybean embryonic axes showed a significant increase in chemiluminescence, which is consistent with increased lipoxygenase activity and $^1O_2$ generation (10) and enhanced oxygen radical production (9). It has been suggested that in some plant species fully functional mitochondria are present in the dry seed (11). The initial lag in oxygen uptake contrasts with the data of Ehrenshaft and Bramble (12), who found a significant increase in the rate of oxygen uptake by maize embryos in the first hour after imbibition. Phytoglobin (Hb) mRNA was detectable on northern blots within 2 h of the onset of imbibition (13). We suggested that the respiration metabolism and oxygen consumption were increased, which was also an important origin of $^1O_2$ because the CL intensity of rice seeds in imbibition were inhibited significantly by rotenone (data not shown). However, $^1O_2$ can be generated through other metabolic pathways in rice seeds during early imbibition, the mechanism of which still needs to be elucidated.

Slawinska *et al.* (3) suggest that CL of imbibing seeds is generated by the processes binding H_2O molecules in the seed coats and/or aleurone layer as well as in the endosperm. Our data suggest that enzymatic processes (i.e. $Q_{10} = 2$) (3) in the range 303–323°K are critical for the generation of excited electronic states which lead to the observed CL. Our previous results (6) showed that rice seeds with a longer storage time had a lower intensity of CL during early imbibition, and the germination rate of rice seeds showed a significant positive correlation with the intensity of spontaneous CL. Our previous report suggested that there was production of active oxygen (6). We believe that the production of active oxygen must be related to enzyme reactions. However, we still cannot identify which enzymes are involved during seed imbibition.

The emission spectrum of intact rice seed, caryopses and seed coat had a greater proportion of red light during early imbibition (see Figs 4–6). Red light is an important emission from $^1O_2$ (14). Cadenas' results showed that low-level chemiluminescence emitted from hepatocytes was at wavelengths beyond 600 nm (i.e. 'red light band') and could be used to monitor the steady-state concentration of singlet oxygen molecular and

provide a useful tool to examine oxygen-dependent radical damage (15).

Compared with the CL of barley (*Hordeum vulgare* L.) and soybean (*Glycine max* L. Merr.) seeds during early imbibition, the CL of rice seeds had a non-linear, logarithmic-like increase of intensity in the range 30–50°C, and a Van't Hoff coefficient $Q_{10} = 2$. The emission spectrum results of whole rice seed, rice and coat had a greater proportion of red light, which led us to the conclusion that the CL of rice seed during early imbibition arises partially from enzyme-catalysed chemical reactions.

In future work we intend to study the effects of specific enzyme inhibitors or activators on CL production.

Acknowledgements

The work was carried out in the International Institute of Biophysics in Neuss, Germany. We thank Professor F. A. Popp and Dr Yan-Yu for their extensive discussions. This work was supported by the Chinese Natural Science Foundation of Guangdong Province (031511), the Science and Technology Project of Guangdong Province, China (2002C20607) and a DAAD scholarship (WLC), Germany.

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