Biochemistry and Biophysics Reports 7 (2016) 20–25

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Monitoring of microwave emission of HRP system during the enzyme functioning

Yu.D. Ivanov [a,*], A.F. Kozlov [a], K.A. Malsagova [a], T.O. Pleshakova [a], S.G. Vesnin [b], V.Yu. Tatur [c], N.D. Ivanova [d], V.S. Ziborov [e]

[a] Institute of Biomedical Chemistry, ul. Pogodinskaya 10, Moscow 119121, Russia
[b] RES Ltd, Moscow, Russia
[c] Foundation of Perspective Technologies and Novations, Moscow, Russia
[d] Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology, Moscow, Russia
[e] Joint Institute for High Temperatures of the Russian Academy of Sciences, Moscow, Russia

ARTICLE INFO

Article history:
Received 1 April 2016
Accepted 5 May 2016
Available online 7 May 2016

Keywords:
Horseradish peroxidase
Microwave emission
Enzyme system

ABSTRACT

Monitoring of microwave emission from aqueous solution of horseradish peroxidase (HRP) in the process of the enzyme functioning was carried out. For the monitoring, a system containing HRP, luminol and H_2O_2 was employed. Microwave emission measurements were carried out in the 3.4-4.2 GHz frequency range using the active and passive modes (active-mode and passive-mode measurements). In the active mode, excitation of the solution in the pulsed electromagnetic field was accomplished. In the passive mode, no excitation was induced. It appears that the passive-mode measurements taken in the course of the peroxidase reaction in the enzyme system have shown a 0.5 °C increase of the microwave signal. Upon the active-mode measurements, taken in the same reaction conditions, the forced excitation of the solution has also led to the increase (by 2 °C) of the level of the microwave signal – i.e. to its 4-fold enhancement compared to the signal obtained in passive-mode measurements.

1. Introduction

HRP pertains to heme-containing enzymes. The study of peroxidases is of great interest due to the fact that these enzymes are well represented in plant and animal tissues [1] and play an important functional role in the organism. Peroxidase catalyzes oxidation of a broad spectrum of organic and inorganic compounds by hydrogen peroxide [2]. Plant peroxidase takes part in the transformation of peroxides and compounds foreign to the organism. The interest in the plant peroxidase is also conditioned by the fact that currently ELISA methods using HRP-labeled chemiluminescent tags for signal amplification have found wide application in analytical biochemistry and diagnostics. ELISA-based analysis of sera from patients with autoimmune thyroid disease, when monoclonal antibodies against TPO conjugated with peroxidase are used, gives an example of such studies [3].

For both plant and animal peroxidases, including heme-containing human peroxidases TPO, LPO and MPO, the peroxidase reaction occurs in a similar way [4]. In general form this reaction can be written as [5]:

$$E + H_2O_2 \xrightarrow{H_2O} \text{Compound I} \xrightarrow{AH_2,\ AH^\bullet} \text{Compound II} \xrightarrow{AH_2,\ AH^\bullet} E + H_2O \quad (1)$$

where AH_2 is a reducing substrate and H_2O_2 is an oxidative substrate.

For HRP-based system peroxidase catalytic cycle is most studied. In practice, luminol, the artificial electron donor ABTS and other substrates are used as reducing substrates for peroxidase [6,7]. Reaction (1) is often used in clinical practice, in biosensors for environmental monitoring and in pharmaceutical therapy [7]. In this reaction luminol is often used to enhance the analysis sensitivity at the expence of chemiluminescence occurring upon the reaction of peroxidase-based catalysis. It is known that the maximum of the chemiluminescence spectrum in the HRP-luminol-H_2O_2 system corresponds to the wavelength ~425 nm [7].

In the present work, the possibility of monitoring the emission from the HRP-luminol-H_2O_2 system not in the visible, but in microwave frequency range, was examined. Measurements were carried out in two modes. Here, they were designated for convenience as the passive-mode and active-mode measurements. The passive mode consists in registration of extremely weak self-radiation of the observation object. In contrast to passive

* Corresponding author.
E-mail address: yurii.ivanov@rambler.ru (Yu.D. Ivanov).

http://dx.doi.org/10.1016/j.bbrep.2016.05.003

radiometry, in the active mode of measurements the object is probed with weak electromagnetic radiation with simultaneous registration of the microwave signal.

Earlier we have shown that microwave emission in the 3.4-4.2 GHz frequency range can be registered in CYP102 A1 containing monooxygenase system which catalyzes fatty acids hydroxylation [8,9]. This system is interesting in that it is a model of cytochrome P450-containing systems, as it contains the heme and flavin domains in one polypeptide chain [10]. That is why this system is often used as an object in enzymatic reaction studies [10–14] and was chosen in the present work to investigate the microwave emission. Besides, in our earlier study on the same it was shown by atomic force microscopy that the functioning of the enzyme is accompanied by fluctuations of the protein globule [12–16]. These fluctuations are able to cause disturbances of aqueous environment of the cytochrome CYP102 A1 molecules thereby generating the microwave emission from the whole solution. We have found that in the case of the heme-containing protein HRP, the fluctuations of the protein globule during its functioning also take place [17]. The amplitudes of height fluctuation of the protein globules of HRP and CYP102 A1 enzymes are equal (~0.8 nm). It was assumed therefore that the functioning of HRP-based system – similar to CYP102 A1-based system - is accompanied by microwave emission. The results of our present study demonstrate that the above-made assumption is correct.

It has been shown that in the HRP-luminol-H_2O_2 system the microwave emission in the 3.4-4.2 GHz frequency range is registered in passive-mode measurements, i.e. in the absence of external electromagnetic field. The influence of the pulsed electric field on the monitoring of microwave emission in the enzyme system (active-mode measurements) was also studied. The expected factor of the pulsed electric field influence was taken into account based on our data on the detection and concentrating of protein upon electrical stimulation [18,19]. As has been shown by us with cytochrome b5 as an example, the excitation of the medium containing this protein in the pulsed electric field with 1 ns front rise time leads to the increase in efficiency of protein detection on the chip surface [19]. In this study, the analogous conditions of measurements were used upon the monitoring of microwave emission from the HRP-luminol-H_2O_2 system to investigate the effect of the pulsed electric field on this system's emissivity.

The measurements were carried out using 10^{-6} M HRP solution. This concentration of the enzyme solution was chosen based on the fact that the concentration of this enzyme in horseradish root is at the same level, $C \sim 10^{-6}$-10^{-7} M [20].

2. Materials and methods

2.1. Reagents

Deionized ultrapure water was obtained using Millipore Simplicity UV system (Millipore, France). Hydrogen peroxide (H_2O_2) and luminol were purchased from Sigma (USA).

2.2. Protein

HRP-C (Sigma, USA) was used throughout. The specific activity of HRP claimed by the manufacturer is 1000 IU/mg. According to [21], HRP exhibits the maximum catalytic activity in the pH range 6.0–6.5. Test aqueous solutions of protein (10^{-6} M and 10^{-9} M) were prepared by dissolving calculated sample of the lyophilized protein in water and following consequent tenfold dilution with deionized ultrapure water.

2.3. Technique of microwave signal monitoring

2.3.1. Catalytic reaction in HRP-based system

The monitoring of microwave emission was carried out in aqueous solution containing reconstructed HRP-based enzyme system containing luminol. For this, 50 μL of reducing substrate (10^{-3} M luminol solution) were added into the measuring cell containing 1150 μL of HRP solution ($C = 10^{-6}$ M or 10^{-9} M); after that, 50 μL of oxidizing substrate (10^{-4} M H_2O_2) were added into the cell. Catalytic reaction in the system occurred according to Eq. (1). Measurements were carried out at $T = 24$ °C for 50–60 min.

In control experiments, the following schemes of addition of the enzyme system's components were used in the above-described conditions:

1) only 50 μL aliquots of water were added to the 10^{-6} M HRP solution;
2) 50 μL aliquot of water and the 50 μL aliquot of 10^{-3} M luminol solution were added to the 10^{-6} M HRP solution;
3) 50 μL aliquot of water and the 50 μL aliquot of 10^{-4} M H_2O_2 solution were added to the 10^{-6} M HRP solution.

2.3.2. Measurement of microwave emission from the solution

Broadband radiothermometer (microwave analyzer) RTM-01 RES, operating in the 3.4-4.2 GHz range, was used as a microwave emission detector. For the measurements, flagpole antenna of microwave analyzer was fully immersed into the sample solution in the measuring cell. The antenna was not removed from the cell throughout the experiment. The addition of solutions into the cuvette and stirring were carried out using automatic pipette.

The data on microwave emission measurements are presented using the brightness temperature (T_{SHF}) units, in which the radiothermometer is calibrated. The measurement accuracy was ±0.1 °C. The measurement data are presented as the T_{SHF} vs time ($T_{SHF}(t)$) dependence.

2.4. Active-mode measurements

The experimental scheme to measure the microwave emission is shown in Fig. 1 and is described in detail in [18]. In the present study this scheme was used with slight modification: the isolation of the HOPG electrode from the reaction medium was achieved by use of mica sheet. For this purpose, 0.25 mm-thick mica sheet was

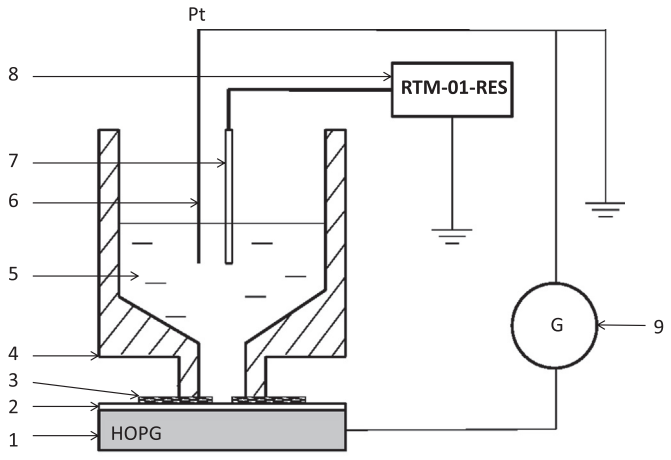

Fig. 1. Experimental scheme for the active-mode measurements of microwave emission from HRP solution. (1) – freshly cleaved HOPG; (2) – mica sheet; (3) – PTFE film; (4) – PTFE cell; (5) – HRP solution; (6) – Pt electrode; (7) – flagpole antenna; (8) - radiothermometer; (9) – pulse generator.