Communications to the Editor

[Chem. Pharm. Bull. 34(4)1834–1836(1986)]

PHOTOACOUSTIC MICROSCOPY FOR THE ANALYSIS OF PEROXIDASE ACTIVITY IN A BIOLOGICAL TISSUE

Katsuya Wada, Tsutomu Masujima, Hisanobu Yoshida* and Hideo Imai[1)]
Institute of Pharmaceutical Sciences,
Hiroshima University School of Medicine,
Kasumi 1-2-3, Minami-Ku, Hiroshima 734, Japan

Peroxidase activity in a microregion was determined by photoacoustic microscopy.

Horseradish peroxidase in a gelatin layer was used as a standard to make a calibration curve coupled with (an enzymatic) staining reaction with 3,3'-diaminobenzidine and hydrogen peroxide. Standard peroxidase activity, 1.6-10 nano-units (95-600 fg), in 20-μm diameter area was determined by the photoacoustic signal from the dye formed.

This method was applied to determine the peroxidase activity in a section of rat small intestine representing a microregional quantitative distribution of the enzyme.The determinations were in good agreement with morphological observations. The total activity of 4.6 micro-units of peroxidase in a section (5.1 x 2.6 mm, 10 μm thick) was determined by integrating the signals from the 20-μm diameter area.

KEYWORDS——photoacoustic microscopy; micro-distribution; peroxidase activity; rat small intestine

Photoacoustic spectrosopy[2)] is based on acoustic (thermal) waves generated by intermittent light absorption and thermal relaxation. It has been applied recently to the analysis of biological tissues.[3,4)] Photoacoustic microscopy (PAM)[5)] with a sharply focused light beam and X-Y scanning of the sample shows the two-dimentional distribution (PAM image) of the light absorbing substances in the sample.

In this paper we describe the use of the PAM method to evaluate the quantitative distribution of the enzyme peroxidase (POD) in biological tissues. POD exists in almost all tissues and is thought to be involved in many important biological processes such as oxidative charge transport.

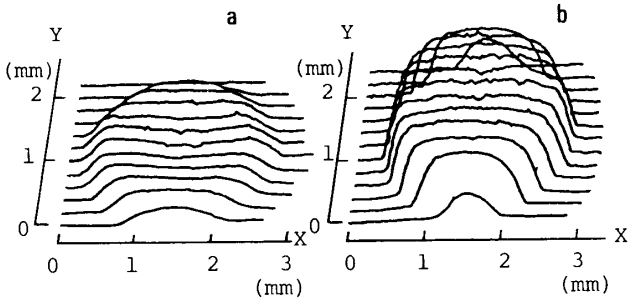

Fig. 1. PAM Images of POD Activities in Gelatin Spots
Horseradish POD activities:
(a) 42 micro-units,
(b) 168 micro-units.

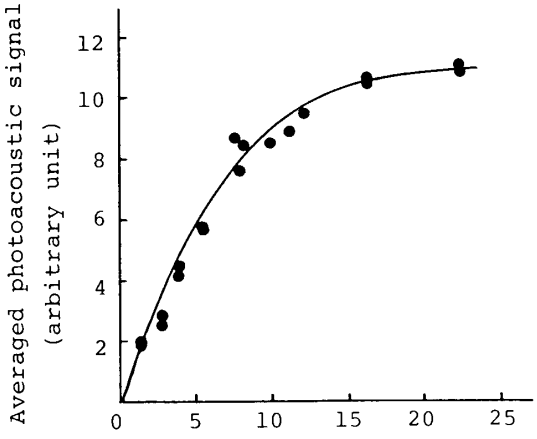

Fig. 2. Calibration Curve of POD Activity

Horseradish POD (Boehringer Mannheim GmbH, FRG) was used as a standard enzyme. A 2.5-μl aliquot of the POD solution (5-134 m-units/ml) mixed with an equal volume of 5% gelatin solution was spotted on a cover glass and dried. The POD spot was fixed in a gelatin layer with 5% glutaraldehyde (4°C, 2 h), then stained with 3,3'-diaminobenzidine (1.4 μmol/ml) oxidized with hydrogen peroxide (19.5 μmol/ml). The spot was incubated for 15 min at room temperature in the presence of Ni^{2+} ions. This enhanced the PAM signal 3 times by changing the stained color from brown to blue black. The reactivity of other enzymes such as catalase etc. was negligible in the present system.

A typical PAM image of a POD spot (ca. 2.5 mm diameter) is shown in Fig. 1. The shapes of the spots were almost uniform, therefore it was possible to determine the POD activity in a 20-μm diameter area by averaging the total PAM signal. A study of the effect of the sample thickness on the signal intensity is in progress.

The relation of the PAM signal to POD activity was curvilinear as shown in Fig. 2. Least-squares analysis of the relation between the logarithmic values of the photoacoustic signal intensity and POD activity gave the following equation,

$$\log[\text{photoacoustic signal intensity}] = 11.9 + 0.816 \times \log[\text{POD activity}] \quad (1)$$

This equation turns out as follows:

$$[\text{POD activity}] = 1.8 \times 10^{-14} \times [\text{photoacoustic signal intensity}]^{1.2} \quad (2)$$

The quantities in brackets are the value of the 20-μm diameter area. The coefficient of variation at ca. 5.5 nano-units/20-μm diameter area was 5.7% (n=5). The exponents in Eq.(2) varied depending on the optical and thermal characteristics of the samples. The reproducibility of the factors is under investigation. At this stage it was difficulty to make a uniform spot of POD activity weaker than 1.6 nano-units/20-μm diameter area, but at this level the S/N ratio by the present method was ca. 50. Pico-units of weak POD activity may be determined provided a uniform spot with such low levels can be obtained.

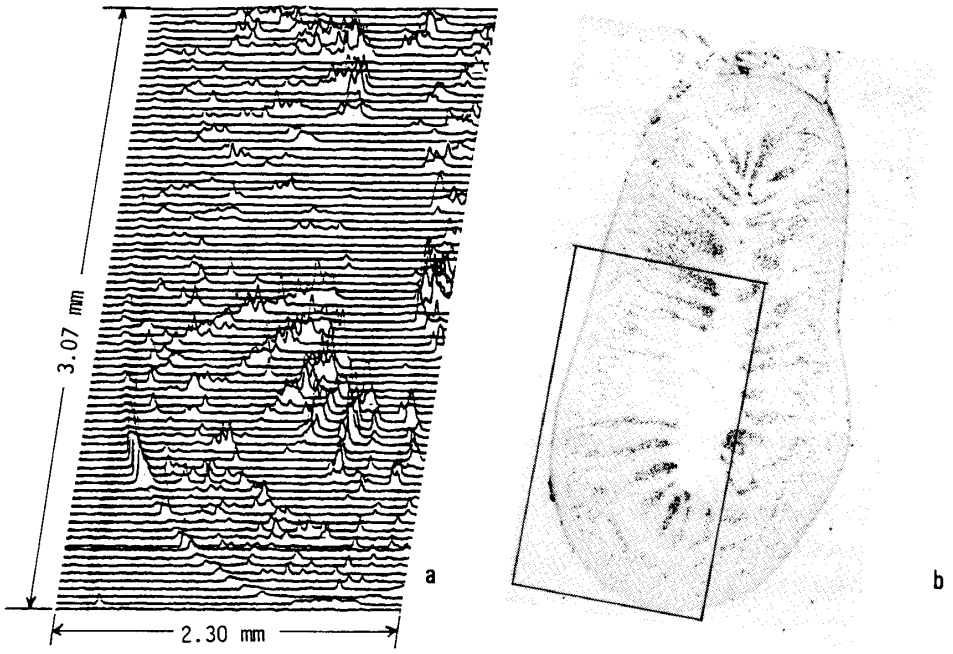

Fig. 3. PAM Image (a) and Photograph (b) of Rat Small Intestine

This method was applied to the determination of POD activity in a section of rat small intestine. A PAM image and a photograph of a typical sample of rat intestine are shown in Fig. 3, which shows POD activity mostly in the mucosal layer and in a blood vessel. By extrapolating the relation of Eq. (2), the POD activity shown in Fig. 3 in the 20-μm diameter area (the sum of several stained particles) was determined to be ca. 0.1-3.0 nano-units. The total activity in the section shown in Fig. 3 (oval shape of 5.1 x 2.6 mm with 10 μm thickness) was determined by the integration of the photoacoustic signals using Eq.(2). The change of scanning axis by 90° gave an error of ca. 5%. Typical variation of the absolute value was 3.9-4.6 micro-units in 4 specimens sectioned successively.

This work was supported in part by a Grant-in-Aid for scientific research, (No. 60571019) from the Ministry of Education, Sciences and Culture, Japan.

REFERENCES AND NOTES

1) Present address: Faculty of Pharmaceutical Sciences, Fukuyama University, Higashimura-cho, Fukuyama, 729-02, Japan.
2) A. Rosencwaig, "Photoacoustics and Photoacoustic Spectroscopy", John Wiley and Sons, Inc., New York, 1980.
3) G. Bults, B.A. Horwitz, S. Malkin and D. Cahen, Biochim. Biophys. Acta, 679, 452 (1982).
4) a) K. Wada, T. Masujima, H. Yoshida, T. Murakami, N. Yata and H. Imai, Chem. Pharm. Bull., 33, 1316 (1985); b) K. Wada, T. Masujima, H. Yoshida, T. Murakami, N. Yata and H. Imai, Chem. Pharm. Bull., 34, 1688 (1986).
5) Y.H. Wong, R.L. Thomas and G.F. Hawkins, Appl. Phys. Lett., 32, 538 (1978).

(Received November 28, 1985)