BIOLOGICAL COMPONENT MICROANALYSIS BY LASER PHOTOACOUSTIC IMAGING IMMUNOASSAY

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Computerized laser photoacoustic microscopy was developed and applied to the immuno-selective imaging of the microdistribution of a biological component. Human lambda-type light-chain was chosen as a model target protein and its peroxidase conjugated antibody was used for selective binding to the target protein and its staining. Using a microscopic calibration curve which was obtained from the images of homogeneously dispersed standard samples, the total quantities of randomly dispersed artificial standard samples were estimated to evaluate the reliability of this method. Finally, the method was applied to a tissue section sample, a human fetal pancreas section, to get the local and total quantitative images of the lambda-type light-chain.

KEYWORDS photoacoustic spectroscopy; photoacoustic microscopy; microscopic analysis; laser; enzyme immunoassay; human lambda-type light-chain

Photoacoustic spectroscopy (PAS) has a potential to be developed as a sensitive and quantitative analytical method[1] and its immunoassay was developed for selective biological component analysis. Filtration of the immuno-precipitate followed by staining was the first photoacoustic immunoassay[2] and Achwall et al. applied PAS to enzyme immunoassay to determine 5-methyl cytosine in DNA.[3] On the other hand, to develop a sensitive analysis for the components in a micro-region, laser photoacoustic microscopy (PAM) was developed and applied.[4,5] In this microscopic determination, the pg order of staining dye was estimated. However, this staining method with dye was not so selective for a particular biological component in tissues. In order to improve the selectivity, enzyme-conjugated antibody was used for selective binding and staining of the target component. A computerized system in our laser PAM improved not only the analysis procedure, but also the quality of the images. Here we report the application of this method.

Human lambda-type Bence-Jones protein (Behringwerke AG, Marburg, W.Germany) which is a principal indication of malignant lymphoreticular disease, was used as a standard and was adsorbed on a 3 mmϕ area of nitrocellulose (NC) membrane filter (TM-2, Toyo Roshi Kaisha, Ltd., Tokyo). The NC membrane was dipped for 30 min in a 5% bovin serum albumin (Sigma Co. Ltd., U.S.A.) in a phosphate buffered saline (PBS) solution to block its remaining adsorbing sites of this membrane. A sample of human fetal pancreas tissue was fixed in a 10% buffered formalin solution and sliced into 5 μm thick sections. The sections were also fixed on a NC membranes. The standard and sample membranes were simultaneously placed in a 1/200 dil. rabbit anti-human lambda-light chain IgG (BioMakor, Rehovot, Israel) solution for 30 min and then in a 1/2000 dil. peroxidase conjugated goat anti-rabbit IgG IgG (BioMakor, Rehovot, Israel) solution for 90 min. The membranes were washed with PBS then placed for 10 min in a

solution of 0.05% 3,3'-diaminobenzidine in Tris buffer (0.05 M, pH7.6) which includes 100 µl of 0.01% hydrogen peroxide and 100 µl of 0.04 M $NiCl_2$ to stain the immuno-reacted area a blue brown color.[6] Light absorption of this color spot on the dried membranes generates heat and this heat induces heat expansion in the surrounding gas phase. The pressure wave thus generated was detected as sound by the He-Ne laser (25 mW) PAM whose system is detailed elsewhere.[7]

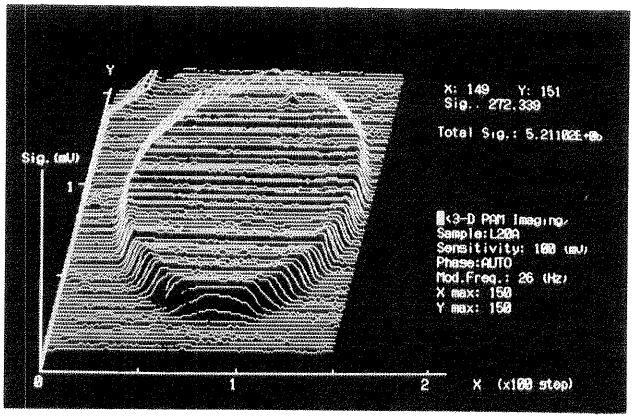

Fig. 1. A Primitive PAM Image of a Standard Spot(3 mmφ) of 20 ng Lambda-Type Bence-Jones Protein

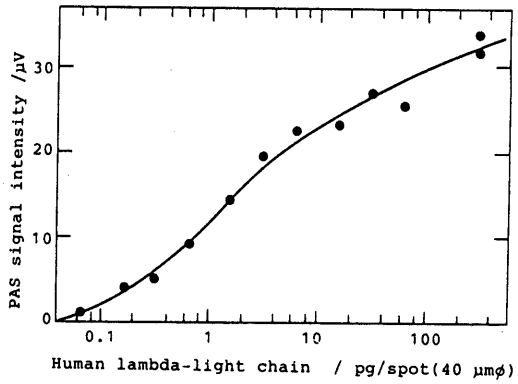

Fig. 2. A Calibration Curve of Lambda-Type Bence-Jones Protein in a Unit Micro-Area

A typical primitive image of a standard spot (20 ng of lambda-type Bence-Jones protein) is shown in Fig. 1. The average value of the background signal in Fig. 1 was subtracted and the signal in the specified area only was accumulated by microcomputer to get the total signal. This total signal was divided by the total steps of laser scanning within the spot area to give an average signal in the one-laser focused micro-area. The calibration curve in the unit micro region (40 µmφ area), thus obtained is shown in Fig. 2. This sigmoid relation is common to the solid phase immunoassay and was fit to the following allosteric function considering the enzyme staining reaction.

$$S = X(1+X)^{A-1}/(L+(1+X)^{A}) \qquad (1),$$

where S is the photoacoustic signal (µV), X is the log(amount of human-lambda light-chain), and A and L are the fitting parameters. The best fit parameters of A and L were obtained by

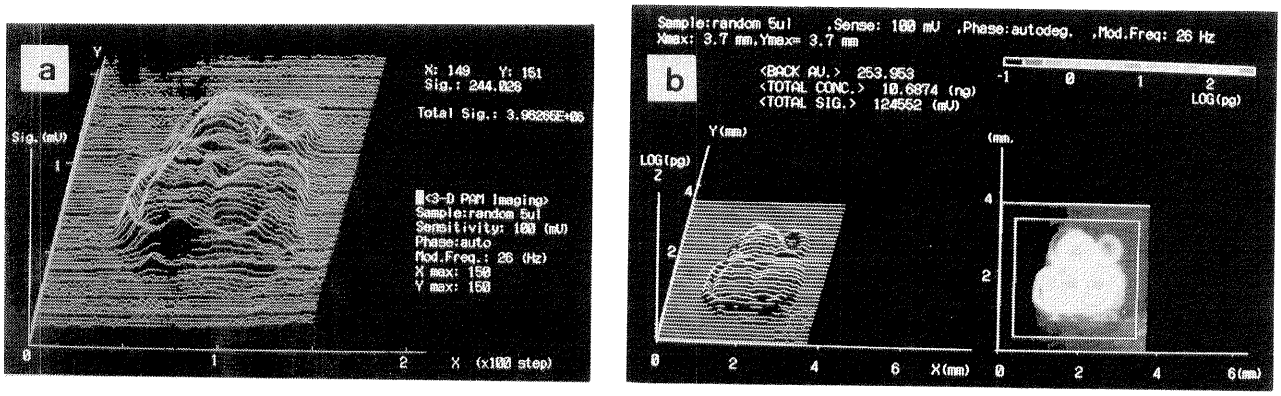

Fig. 3. A Primitive PAM Image of an Artificially Made Randomly Dispersed Spot of Lambda-Type Bence-Jones Protein (Total 10 ng) (a) and Its Analyzed Quantity Image (b)

the least squares method. The coefficient of variation (within-run) was 4.7% (n = 5) at 3.25 pg/spot. The signal amplitude at each spot was fit to this curve and the quantity was estimated. Thus a signal image can be converted into a quantity image by calculation using this computerized system.

In order to check the performance for quantitative analysis, artificially made randomly dispersed spots were analyzed by this method based on the calibration curve. Fig. 3(a) shows an example of a primitive image of this sample and Fig.3(b) is its quantity image whose z-axis is the amount of lambda-type light-chain. Randomly dispersed samples of lambda-type Bence-Jones protein, 10 ng in total, were finally estimated to be 10.7 ng (this case), 8.1 ng, and 13.9 ng. This may be acceptable for this accumulation analysis by the complicated procedures.

This method was applied to a tissue section of a human fetal pancreas section as shown in Fig. 4. The local amount of 0.1-10 pg of lambda-type light-chains are located in the area of 40 μmφ. The total amount of the protein in the outlined square area can be estimated to be 11±2 ng. The immunoassay is much more specific than the staining dyes and the enzymatic staining also enhances the sensitivity. The cross reactivity of the antibody was not taken into account. So in this case, the stained protein is not always the lambda-type Bence- Jones protein (free lambda-type light-chain) but also the the lambda-type light-chain in the IgG. Use of a monoclonal antibody and cross- and multi-item immunoassay are under development.

This immunoreactive determination in a micro region seems to have advantages in its stable staining (no signal variation on the laser irradiation) and sensitivity (due to enzymatic reaction and photoacoustic detection) which are superior to the fluorescence imaging method which suffers from the decreasing fluorescence intensity on the excitation-light irradiation. The present disadvantages of this method are the adaptability only for dried samples in the final stage and the tens of minutes of measurement time.

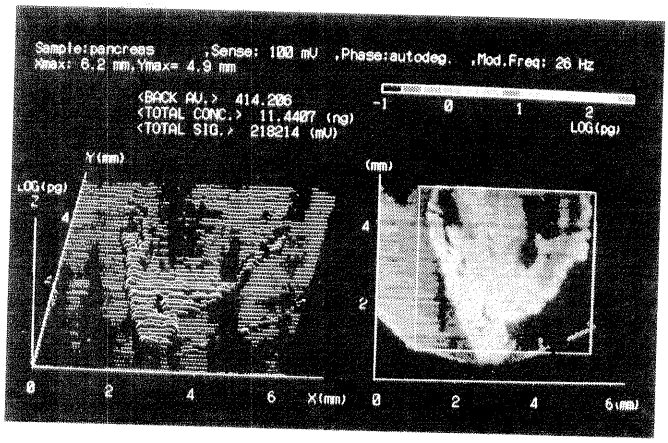

Fig. 4. A Quantitative Image of a Lambda-Type Light-Chain in a Human Pancreas Tissue Section (5 μm Thick) Analyzed from Its Primitive PAM Image

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