Evaluation of Avidin-Biotin Binding Using a Biotin Labeled with Daunomycin by Photoacoustic Spectrometry

Kazuharu Sugawara, Yuuki Yamaguchi, Masaharu Nago, Masami Futamata, Suwaru Hoshi, and Kunihiko Akatsuka
Kitami Institute of Technology, Kitami, Hokkaido 090-8507

(Received March 19, 1998; CL-980205)

A method of evaluation for avidin-biotin binding using photoacoustic spectrometry (PAS) was developed. Biotin labeled with daunomycin was prepared. The avidin-biotin binding between avidin and the labeled biotin (LB) makes avidin colored. This is because the part of daunomycin is taken in biotin sites of avidin. Therefore, the concentration of avidin could be estimated based on measurement of the LB with avidin by PAS.

Biotin is well known as a vitamin H. Roles of biotin in living body are growth factor and co-enzyme of carboxylase. It also relates to the metabolisms of amino acid and sugar. Biotin selectively combines with avidin that is a protein. The avidin-biotin binding is one of the strongest bindings between protein and ligand. Therefore, studies of the avidin-biotin binding have been reported,[1, 2] and assays of avidin and biotin have been investigated. We also proposed several methods to evaluate the avidin-biotin interaction by an electrochemical procedure.[3, 4]

This paper describes the application of PAS to estimate the avidin-biotin binding. The PAS has been used for the determination of metal ions using complex formation[5], evaluation of powder materials[6] and semiconductor.[7, 8] However, study of the binding between the protein and ligand is few. The principle was as follows. Biotin labeled with daunomycin having visible spectra is prepared. When the LB combines with avidin, the part of daunomycin is taken in binding site to biotin of avidin. Although avidin is usually colorless in a solution, avidin becomes colored on the basis of the binding. After the separation of LB with avidin (LB Bound) and LB without avidin (LB Free) by thin layer chromatography, each species on the TLC sheet is directly measured without pretreatment. Consequently, the avidin-biotin binding can be evaluated by the change of the photoacoustic signal of the LB, and avidin is detected. In addition, length of spacer between the label and the biotin moieties that influenced the binding was investigated. A merit of this method is that both LB Bound and LB Free are monitored. Enzyme-linked ligand sorbent assay (ELLSA) only measures LB Bound.[9] This is because activity of enzyme binding on well is determined. Furthermore, ELLSA needs a number of reagents, more time and sample volume comparing to this method.

Sulfosuccinimidyl D-biotin, sulfosuccinimidyl N-(D-biotinyl)-6-aminohexanoate, sulfosuccinimidyl N-[N'-(D-biotinyl)-6-aminohexanoyl]-6'-aminohexanoate as biotinylation reagents, avidin and biotin were supplied from Wako Pure Chem. Co. (Osaka, Japan). Daunomycin was purchased from Sigma. Preparation of the LB was described in ref. 3. Since the visible spectrum of the product solution agreed with the spectrum of daunomycin the concentration was determined by the absorbance at 490 nm. Daunomycin bound with Biotin-Sulfo-Osu, Biotin-AC_5-Sulfo-Osu and Biotin-$(AC_5)_2$-Sulfo-Osu are called S-LB-D, M-LB-D and L-LB-D, respectively (Figure 1). The structure of the LB was decided on the basis of the NMR data.[3]

Figure 2 shows the diagram of the PAS employed in this study. An Ar (GL G 3202, NEC Co.) at 514.5 nm having power of 1 W

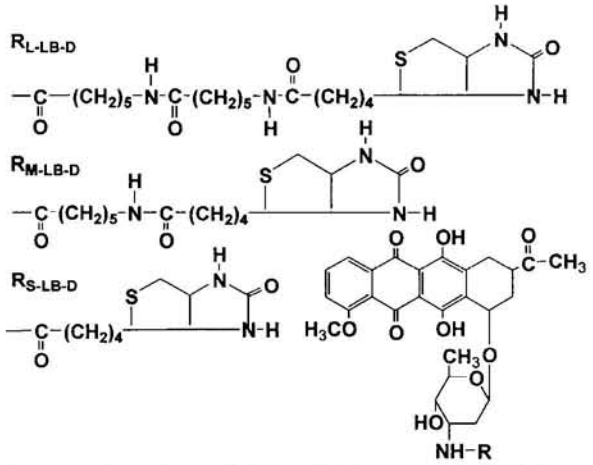

Figure 1. Structures of biotin with Daunomycin(LB-D).

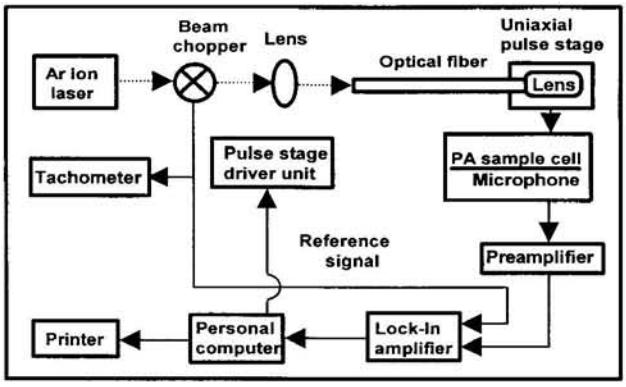

Figure 2. Diagram of photoacoustic spectroscopy system.

was used as a light source. Daunomycin was selected to have maximum wavelength at 490 nm. Chopper (40 r.p.s) was two wing of aluminum coated with black paint. Laser light was irradiated to the photoacoustic cell through optical fiber (4 mm in diameter, 10 m for length) and moved by stage with mortar. PAS signal was detected by microphone (UC-11A, RION) and amplified with a preamplifier (NA-57, RION). Next, the signal was send to lock-in amplifier (Model 5600A, NF ELECTRONIC INSTRUMENTS). The signals were recorded by a personal computer (NEC) with an A/D converter

Avidin and LB were incubated in 0.1 mol dm^{-3} phosphate buffer at pH 7 for 60 min. Total volume was 40 μl. A 5 μl portion of the resulting solution was spotted on TLC plate (silica gel). Development of the chromatogram was carried out with a solvent mixture of ethanol-ammonia (4:1). Thin layer chromatograms were shown in Fig. 3. For this solvent, avidin does not move from point spotted, and the LB Free develops with the solvent. Accordingly,

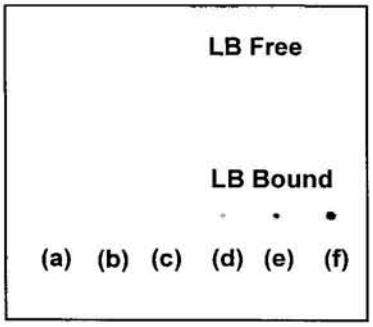

Figure 3. Thin-layer chromatograms for S-LB-D and avidin. Conecentration of avidin/ 10^{-4} mol dm^{-3}, (a) 0.44, (b) 0.88, (c)1.75, (d) 3.50, (e) 5.25, (f) 10.5, $2x10^{-5}$ mol dm^{-3} S-LB-D, Reaction time=60 min, Developing solvent: C_2H_5OH - NH_3 (4:1(v/v)).

LB Bound can be separated from the LB Free. The spot of LB Bound became dark with increasing the concentration of avidin. The binding of avidin and the LB was observed visually.

The TLC plates were provided for the measurement by cutting out 10x10 mm squares containing the spots and placed on holder of the PAS cell. Photoacoustic signal of S-LB-D is shown in Fig. 4. A signal based on absorption of the LB appeared. Signal of LB is obtained by subtracting the PAS intensity from the corresponding intensity of silica gel substrate. Similar signals were also obtained for another LBs. Figure 5 shows the change of the signal of LB as a function of the concentration of avidin. The signal of LB bound increased with increases in the concentration of avidin. When a sufficient amount of avidin was added to bind all LB, the signal became constant. In contrast, the signal of LB Free decreased. Species on the TLC plate can be measured in 10^{-10} g order by photoacoustic spectrometry directly, and the avidin-biotin binding was monitored. The detection limit of avidin using L-LB-D, estimated as three-times of the standard deviation of the blank, was $2x10^{-6}$ mol dm^{-3}. The relative standard deviation at $2x10^{-5}$ mol dm^{-3} biotin was 5.5%(n=5). The change of intensity of L-LB-D is small, however it combines with avidin in the low concentration comparing to S-LB-D. The binding of M-LB-D for avidin is between L-LB-D and S-LB-D. Accordingly, it was found that the apparent strength of the binding depended on the length of the spacer. This reason is that the binding site of avidin for biotin has a hydrophobic environment, while the surface of the avidin molecule is hydrophilic. Hence, the hydrophobic property of the spacer contributes to the strength of the binding in addition to the avidin-biotin interaction. A steric hindrance was also due to a sugar part of daunomycin relating to the binding. In a previous study, the electrochemical assay of avidin was carried out, where the strength of the binding was M-LB-D>L-LB-D>S-LB-D.[4] This suggests that when avidin binds with M-LB-D and L-LB-D, the electroactive the avidin-biotin interaction. In addition, it is expected that a steric hindrance due to a sugar part of daunomycin relating to the binding. In a previous study, the electrochemical assay of avidin was carried out by biotin with daunomycin, where the strength of the binding was M-LB-D>L-LB-D>S-LB-D.[4] This suggests that when avidin binds with M-LB-D and L-LB-D, the electroactive part is not completely covered with avidin. LB Bound has electroactivity because daunomycin is located far from the binding site of avidin owing to the presence of a longer spacer. Therefore, it was expected that order of PAS was different from that of the electrochemical

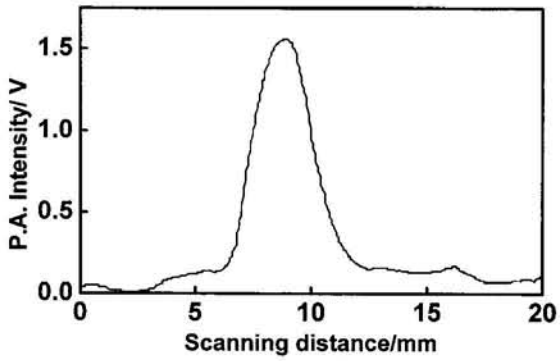

Figure 4. Photoacoustic signal of S-LB-D Free on a silica gel plate. $2x10^{-4}$ mol dm^{-3} S-LB-D Free,1. $1x10^{-4}$ mol dm^{-3} avidin, Reaction time=60 min, Developing solvent : C_2H_5OH-NH_3 = 4 : 1 (V/V).

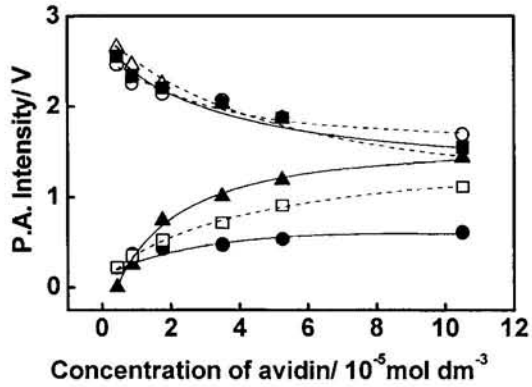

Figure 5. Binding between LB-D and avidin.● L-LB-D Bound, ○ L-LB-D Free, □ M-LB-D Bound, ■ M-LB-D Free, ▲ S-LB-D Bound, △ S-LB-D Free Various concentrations of avidin and 1.9 $x10^{-4}$ mol dm^{-3} LB-D were incubated for 60 min.

assay. Difference of signal intensity obtained by PAS may depend on degree covering of daunomycin.

The evaluation of the interaction between avidin and biotin was demonstrated by the photoacoustic method. In future, affinity constant between LB-D and avidin will be estimated. Furthermore, detection of biotin will be investigated by using competitive reaction of LB-D and biotin to avidin.

References and Notes

1 M. Wilchek and E. Bayer (EDS.), Methods in Enzymology, **184,** Academic Press, 1990.
2 N. M. Green, L. Konieczny, E. T. Toms and R. C. Valentine, *Biochem. J.*, **125**, 781 (1971).
3 K. Sugawara, S. Tanaka and H. Nakamura, *Anal. Chem.*, **67** , 209 (1995).
4 S. Tanaka, F. Yamamoto, K. Sugawara and H. Nakamura, *Talanta*, **44** 357(1997).
5 S. Ikeda, Y. Murakami and K. Akatsuka, *Chem. Lett.*, **1981,** 363.
6 C. Q. Yang and W. G. Fateley, *J. Mol. Struct.*, **146**, 25 (1986).
7 K. Kubato and H. Murai, *J. Appl. Phys.*, **56**, 835 (1984).
8 H. Yamamoto, I. Suemune and M. Yanishi, *J. Appl. Phys.*, **60,** 2621 (1987).
9 S. Tanaka, T. Takeuchi and G. Rechnitz, *J. Chromatogr.*, **597**, 443 (1992).