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Evaluation of detectable depth on SH-SAW biosensor using antibody, antigen, and secondary antibody complexes

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***Abstract*— A shear horizontal surface acoustic wave(SH-SAW) based sensor measures multiple physical quantities of liquid specimens at the same time. Taking advantage of this measurement feature, SH-SAW sensors have been applied to wide varieties of sensors. SH-SAW biosensor is a form of SH-SAW sensor applications. SH-SAW biosensor detects immunoreactions occurring on its sensing area as velocity and/or amplitude changes of SH-SAW. Besides, SH-SAW biosensor has a sensing property determined by depth of sensing area, which is called a viscous penetration depth. However, previous studies have neglected structures of measuring objects. In other words, mass of detecting objects concentrate on sensing area. In order to clarify sensing properties in height direction of SH-SAW biosensor, we measured two different size of proteins, one is a C-reactive protein, CRP, and the other is a conjugate of CRP antigen and anti-CRP antibody. The results of the experiments showed a different velocity-amplitude change ratio in each molecular size. As the amplitude change of SH-SAW shows viscosity of specimen, this is considered to be reflected size difference of molecule. This result is considered to be suggested a structure analysis potential of SH-SAW biosensor.**

Keywords—SH-SAW, Biosensor, Viscous penetration depth, Antigen-antibody reaction

I. Introduction

A shear horizontal surface acoustic wave(SH-SAW) has a characteristics that its displacement exists within propagating surface, as such, its energy does not diffuse perpendicular direction into adjacent medium. Taking advantages of this propagating characteristics, SH-SAW has been used in detection principles of liquid sensors[1]. On the other hand, because propagating characteristics of SH-SAW are affected by multiple physical quantities of adjacent medium, such as density, viscosity, permittivity, and conductivity, SH-SAW based liquid sensors have been used in wide variety of sensing applications[2-3]. SH-SAW biosensor is a form of SH-SAW based liquid sensor applications[4]. SH-SAW biosensor measures an amount of antigen-antibody reactions occurring on immobilized molecules (antibodies) which are preliminarily immobilized on own sensing area, and bind specifically with individual target substances (antigens). Once antibodies immobilized on the surface bind their targeting antigen, velocity and/or amplitude of SH-SAW are changed in accordance with amount of bound antigens. The combination with propagating characteristics of SH-SAW and antigen-antibody reaction enables real-time quantitative measurement of biological reactions without the use of label substances. Additionally, SH-SAW detects not only contacted substances onto the sensor surface, but also properties of medium distant from the sensor surface, because SH-SAW displacement is penetrated into medium above sensor surface. This characteristics is brought by a viscous penetration depth. The displacement penetration depth, denoted δ, is determined by the viscosity, η_{liq}, and density, ρ_{liq}, of the antigen diluting solvent medium, such as normal saline solution, plasma, serum, and so on, and angular frequency of sensor device, ω, which is expressed as equation 1.

$$\delta = \sqrt{\frac{2\eta_{liq}}{\rho_{liq}\omega}} \tag{1}$$

However, previous studies have not taken account this height directional sensing properties of SH-SAW into immune reaction detection. In other words, previous studies have treated measuring antigens and antibodies as almost like a volume less objects. Considering height directional information against measured results corresponds to reflect the structure of antibodies and antigens. Thus, measuring effects by height directional structures could bring a new way to analyze antigen-antibody reaction from another perspectives.

II. Materials and Methods

In order to investigate the height directional measurement characteristics of SH-SAW biosensor, which is brought by viscous penetration depth δ, two different sizes of molecules were measured using two SH-SAW sensor devices whose operating frequencies are in integral multiple relationship.

A. Measured Molecules

In a series of evaluation, two different sizes of molecules, C-reactive protein antigen(CRP-Ag) and antibody(CRP-Ab), were chosen to be measured immune reactions on the SH-SAW sensor device. The CRP is a protein found in blood plasma, whose circulating concentrations increase with corresponding to inflammation in the body. For this property, CRP is applied as clinical marker for acute and chronic inflammation[5]. Furthermore, as a physical characteristics, CRP-Ag is a pentamer which is composed of five identical subunits. Molecular size of CRP-Ag is around 11nm in diameter and 118kDa in weight[6]. In this evaluation, immunoglobulin G(IgG) classified monoclonal antibodies were used as CRP-Abs. Molecular size of IgG is 10-15nm in height and 150kDa in weight[7-8].

B. Sensor Devices

In order to investigate height directional sensing characteristics of SH-SAW biosensor, 250MHz and 500MHz operating SH-SAW sensor devices were prototyped using quartz substrate. The devices were designed using perfectly shared parameter normalized with wavelength. In this way, measurement characteristics differences depending on the viscous penetration depth can be observed, because viscous penetration depth is determined with angular frequency of sensor device. Schematic illustration and their design parameters of prototyped devices are show in Fig.1 and Table I. Sensing area where is SH-SAW propagating back and forth, is coated with detecting antibodies for CRP-Ag and CRP-Ab via crosslinking reagent(Crosslinker).

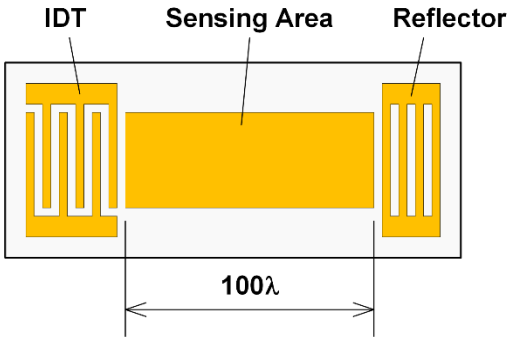

Fig. 1. Schematic illustration of prototyped SH-SAW biosensor

TABLE I. DESIGN PARAMETER OF PROTOTYPED SH-SAW BIOSENSOR

Item	Operating Frequency	
	250MHz	*500MHz*
Wavelength	20μm	10μm
Sensing Length	4000μm (200λ)	2000μm (200λ)

C. Experimental Procedure

SH-SAW biosensor sensing characteristics in height direction were evaluated by measuring velocity change and/or amplitude change of SH-SAW caused by immune reaction occurring on its sensing area. In the evaluation process, CRP-Ag and CRP-Ab were applied onto sensing area and measured each SH-SAW propagation characteristics changes separately, and in series. CRP-Ag concentration was varied, 0.3, 0.6, 1.2, 2.4, and 4.8 μg/ml. Also, a concentration of CRP-Ab was fixed at 120μg/ml. CRP-Ab functions as secondary antibody(2Ab) and forms antigen-antibody complexes. Before and after each measurement, specimen was replaced with buffer solution to be set signal standards. In this evaluation, we used Tris-Buffered Saline with Tween(TBST) as buffer solution. After a round of measurement, sensor devices were regenerated by applying HCl; an extreme pH value change, from pH: 7.6 to pH: 1, forces surface immobilized antibodies to release their captured antigen-antibody complexes. Sensing area surface image during measurements are shown in Fig.2.

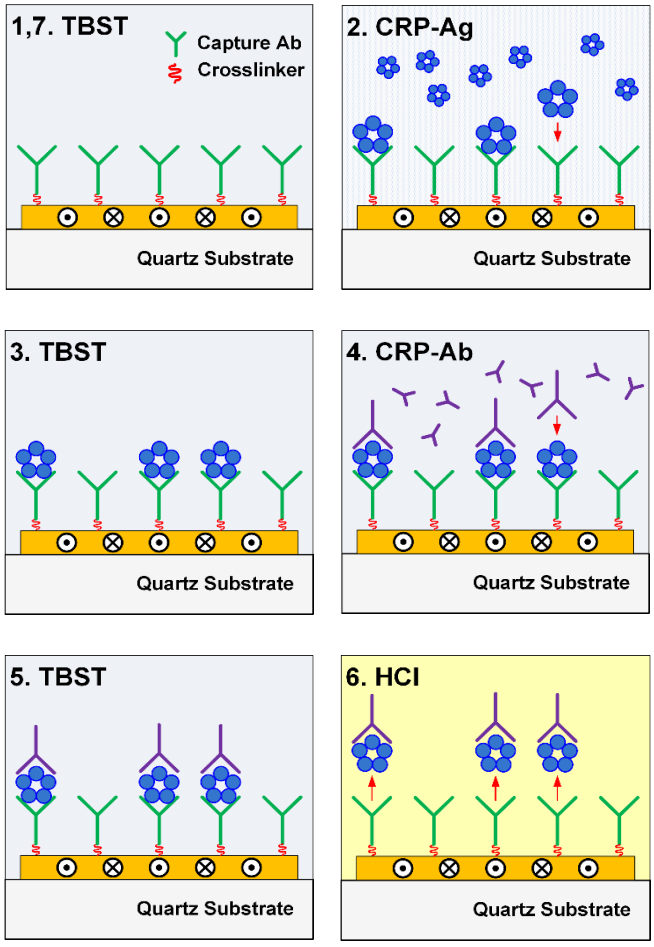

Fig. 2. Sensing Area Surface Images during Measurements

TABLE II. MEASUREMENT PROCESS

Step#	Reagents	Reaction Time
1	TBST	1min.
2	CRP-Ags	3min.
3	TBST	1min.
4	CRP-Ab	3min.
5	TBST	1min.
6	HCl	2min.
7	TBST	1min.

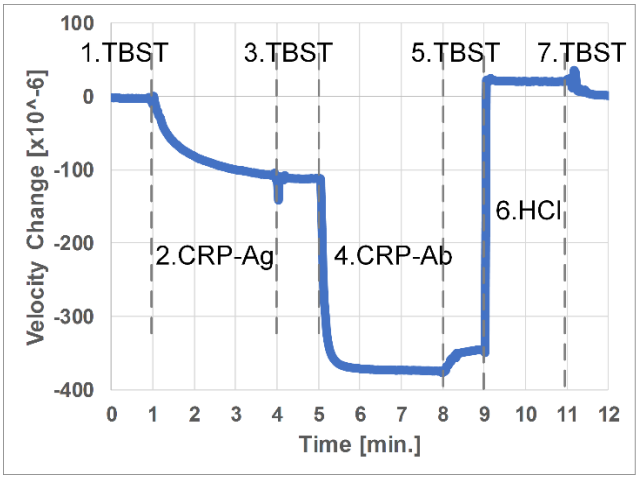

Fig. 3. SH-SAW Velocity Change (CRP-Ag: 4.8mg/ml, 250MHz)

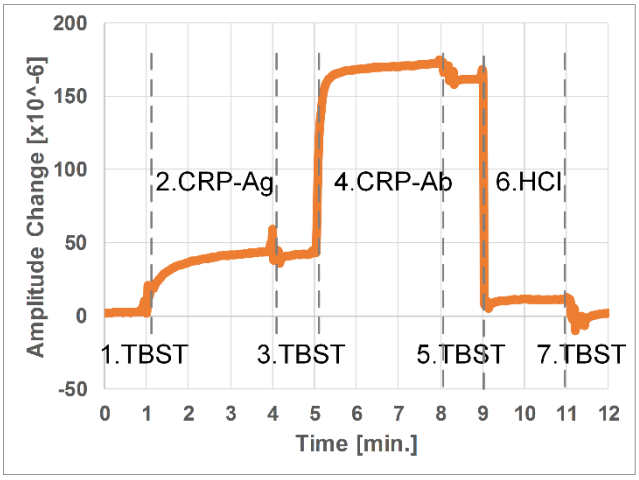

Fig. 4. SH-SAW Amplitude Change (CRP-Ag: 4.8mg/ml, 250MHz)

III. RESULTS AND DISCUSSIONS

Immune reactions have been measured with varying CRP-Ag concentration. Measurement process is shown in table II. Fig. 3 and 4 show recorded typical velocity and amplitude changes, here is posted data of 4.8μg/ml CRP-Ag concentration measured with 250MHz device as an example. In order to eliminate a viscosity effect of dilution buffer and interference effects from suspending unreacted specimen residues, amounts of velocity change and amplitude change were defined by difference of TBST readouts between before and after of target specimen measurement steps. Measured velocity and amplitude changes resulting from immune reactions on the sensing area are shown in Tables III and IV by device operating frequency. With no relations to sensor device operating frequency, both results show that velocity decreased with CRP-Ag concentration. Concentration of CRP-Ab was set constant at 120μg/ml, but its velocity changes were correlated with CRP-Ag concentration, because CRP-Ab specifically reacts captured CRP-Ag in the previous step. As is case with velocity change, also amplitude decreased with CRP-Ag concentration; the amplitude change is defined decreasing with positive. These data are plotted onto the velocity-amplitude plane, Figs. 5 and 6. The graphs show that fitting lines for CRP-Ab have higher slope compare than that of CRP-Ag. A perturbation theory analysis applied on a model which is consisted of SH-SAW sensing area covered with thin metal film indicates velocity is changed with deposited metal film thickness. On the other hands, also, perturbation theory indicates amplitude is not changed with film thickness[9]. The experimental results suggest that surface immobilized antibodies and their captured CRP-Ags and CRP-Abs do not behave like deposited thin film; formed complexes can be estimated to have a three-dimensional structure. The fitting line slope difference between CRP-Ag and CRP-Ab is considered to be reflected molecular size difference, CRP-Ag(15nm+11nm) and CRP-Ab(15nm+11nm+15nm). Also, difference of fitting line slope between 250MHz and 500MHz sensor device is considered to be caused by difference of viscous penetration depth determined by operating frequency.

TABLE III. MEASURED RESULTS (250MHz)

CRP-Ag [μg/mL]	CRP-Ag [x10^{-6}]		CRP-Ab [x10^{-6}]	
	Velocity	*Amp.*	*Velocity*	*Amp.*
0.3	-17.2	4.1	-24.8	20.5
0.6	-26.9	9.5	-47.8	38.0
1.2	-52.3	20.5	-100.3	69.8
2.4	-84.6	31.1	-178.9	104.2
4.8	-109.0	38.8	-233.5	120.1

TABLE IV. MEASURED RESULTS (500MHz)

CRP-Ag [μg/mL]	CRP-Ag [x10^{-6}]		CRP-Ab [x10^{-6}]	
	Velocity	*Amp.*	*Velocity*	*Amp.*
0.3	-19.5	28.3	-64.9	53.2
0.6	-53.1	49.3	-96.2	96.6
1.2	-91.8	49.3	-195.8	134.4
2.4	-120.8	48.2	-300.5	190.0
4.8	-149.1	56.0	-316.1	195.1

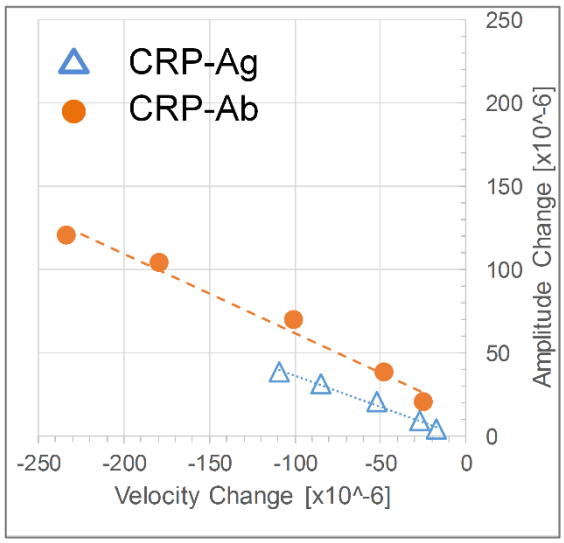

Fig. 5. Measured Velocity and Amplitude Change 250MHz

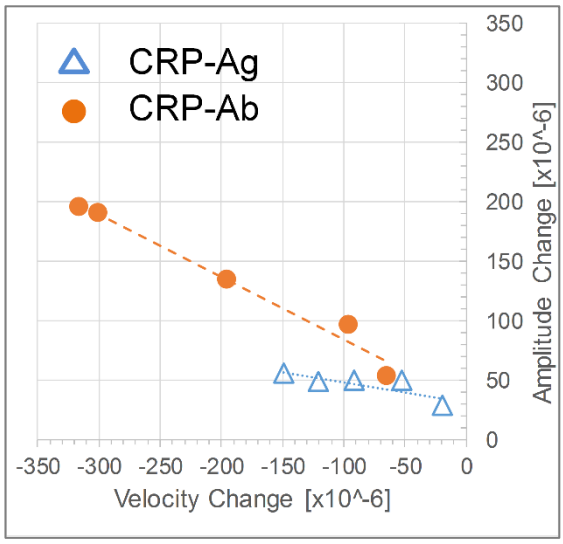

Fig. 6. Measrued Velocity and Amplitude Change 500MHz

IV. Conclusion

The results from a series of experiments showed a different fitting line slope on the plotted plane of velocity-amplitude change with respect to measured molecular size. According to considerations with combined perturbation theory and the series of experimental results, SH-SAW biosensor exists different behavior depending on measuring molecular size. These results are considered to suggested that SH-SAW biosensors capable of analyzing molecular structures.

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