Surface Acoustic Wave Biosensors for the Quantification of TNF-α/SPD-304 Interaction

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Surface Acoustic Wave Biosensors for the Quantification of TNF-α/SPD-304 Interaction

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Abstract

In this study, a surface acoustic wave (SAW) biosensor has been investigated to quantify the affinity and to estimate the binding kinetic between tumor necrosis factor α (TNF-α) and its inhibitor: SPD-304. To the best of our knowledge this is first ever report on real time and label free monitoring of TNF-α/ SPD-304 affinity.
Effects of the SPD-304 solvent and DMSO cosolvent on TNFα/SPD-304 interaction have been investigated. Gravimetric results indicate that the limit of detection (LOD) of the developed biosensor was of order of 10 nM and that DMSO cosolvent influences the kinetic of interaction, the saturation value, and thus the value of the dissociation constant (K_d) of the TNF-α / SPD-304 system.

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Keywords: SAW biosensor ; TNF-α ; SPD-304 ; Affinity ; DMSO cosolvent

1. Introduction

Tumor necrosis factor dysregulation is implicated in several pathological conditions, including cancer, diabetes, and auto-inflammatory diseases such as rheumatoid arthritis and Crohn's disease [1]. As of today, only few small molecules antagonizing directly TNF-α have been described such as polysulfonated naphthylurea suramin and its

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analogues [2-3], and SPD-304, an indole-linked chromone, that is suspected of dissociating the TNF-α trimers and, therefore, blocks the interaction of TNF-α with its receptor [4].

Binding affinity is one of the key elements to appreciate intermolecular interactions driving biological processes, structural biology, and structure-function relationships. It is typically determined by the equilibrium dissociation constant (K_d), which is used to evaluate and rank order strengths of bimolecular interactions. Many techniques, such as ELISA, gel-shift assays, pull-down assays and fluorescence permit to estimate the dissociation constants. However they are time consuming and require labeling of the analytes of interest. Piezoelectric devices, such as quartz crystal microbalances and surface acoustic wave devices, are precious tools for investigation and quantification of binding strengths between biological molecules in general, and proteins-ligands in particular. They could also gain information about the kinetics of recognition and dissociation. Moreover, they are sensitive, selective, and can measure K_D values in the nanomolar range [5-9].

In this study, we have investigated a surface acoustic wave (SAW) biosensor to quantify the TNF-α/SPD-304 affinity and to estimate the binding kinetic of SPD-304 to TNF-α, in presence and in absence of dimethyl sulfoxide (DMSO) cosolvent.

2. Materials and methods

The developed SAW sensor consists of a dual delay lines fabricated on a 36° $LiTaO_3$ piezoelectric substrate. As the interdigital transducer (IDTs) were patterned with a periodicity of $\lambda = 40$ µm, the operating frequency was of order of 104 MHz. The measurement setup consists of a SAW sensor, a Kalrez® flow cell, deposited on the sensing region, a PMMA cover including inlets and outlets connected to a peristaltic pump, and a homemade pulse mode system with two independent paths. The measured parameter is phase and/or amplitude shifts of the sensing line according to the reference one [10]. Before any experiment, SAW devices were immersed for 30 min into a piranha solution (1:1 (v/v) 98% H_2SO_4/30% H_2O_2). They were then rinsed with ultra-pure water and dried. For all biosensing experiments, the buffer solution was HBS-E (pH 7.4) containing 10 mM HEPES, 0.15 mM NaCl and 3 mM EDTA.

3. Biosensor development

TNF-α was first dissolved in HBS-E (pH 7.4), at a concentration of 1 µg/ml , before being deposited during 2 hours on the gold sensing area of the SAW device to form the recognition layer of this biosensor. Like other thiol containing proteins (e.g., BSA), TNF-α may have the capacity to bind covalently to the surface of gold through the S–Au bonding. However, evidence to confirm this binding mechanism is lacking and a target of current research [11]. HBS-E solution is then brought on the sensing area using the peristaltic pump. When phase stability is reached, SPD-304 is injected in the flow cell. A subsequent rinsing with HBS-E permits to remove non fixed molecules.

4. Results

TNF-α / SPD-304 recognition was investigated using SAW-sensor, by recording the output signal's phase change versus time (Fig. 1a). In a first experiment, SPD-304 was dissolved, at a concentration of 10 mM, in HBS-E buffer containing 5% dimethyl sulfoxide (DMSO), an organic cosolvent generally used to solubilize compounds in aqueous buffers. These experimental conditions are comparable to the optimum ones determined in the previous study of Papaneophytou et al [12]. Gravimetric results, presented in Fig. 1a, show two steps recognition: a rapid one, with a time constant of (41 ± 9) s, followed by a slow one with a characteristic time constant of about (323 ± 4) s. This behavior indicates the possible presence of two binding sites, while the previous cited study suggests the existence of only one SPD-304 binding site per protein molecule [12].

The SAW-sensor's response according to SPD-304 concentrations is reported in Fig. 1b. The best fit of experimental values was obtained with a two binding sites model, confirming the previous hypothesis. The first dissociation constant, $K_d = 8.9 \pm 3.3$ µM, is consistent with that obtained by Papaneophytou et al

(K_d = 5.36 ± 0.21 µM) [12]. The second one, K_d = 0.86 ± 0.42 mM, is meanwhile almost a hundred times greater than the first one.

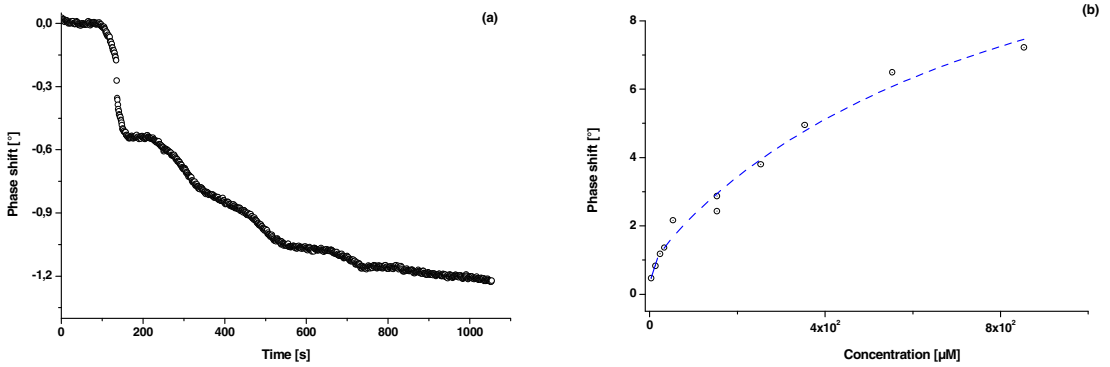

Fig. 1: SAW sensors' response in presence of DMSO (a) versus time and (b) towards SPD-304 concentration.

In a second experiment, SPD-304 was dissolved in HBS-E buffer without DMSO, as this organic cosolvent can reduce the average charge and could possibly destabilize protein–ligand interactions. The SAW sensor's response according to time and to SPD-304 concentration, are presented in Fig. 2a and Fig. 2b respectively.

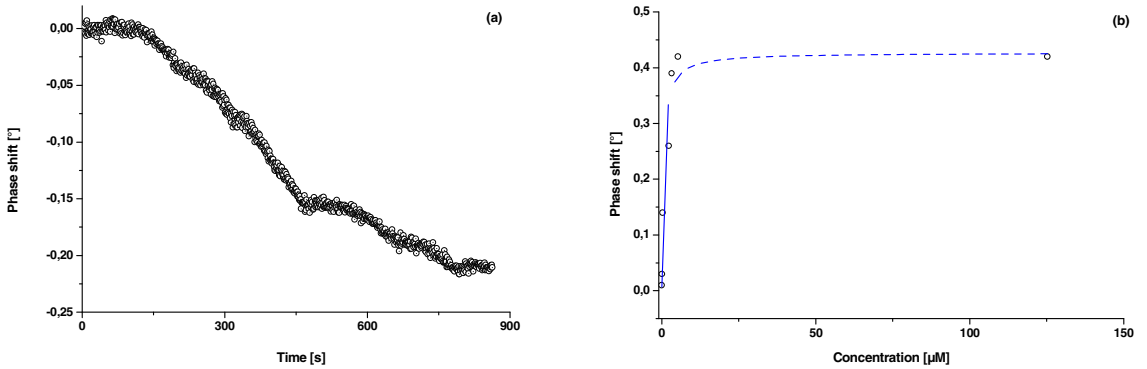

Fig. 2: SAW sensors' response in absence of DMSO (a) versus time and (b) towards SPD-304 concentration.

Compared to Fig. 1a, Fig. 2a shows that only one SPD-304 binding site per TNF-α is discernible (even if a slight slope change is observed). The corresponding time constant, of order of 516 ± 15 s, indicates that the non-use of DMSO slows down the kinetic of the recognition between the investigated ligand and the protein. We noticed also a net diminution of the saturation value (Fig. 2b).

The dissociation constant, determined from fitting of the experimental data of Fig. 2b, was found of order of (0.6 ± 0.3) µM. The limit of detection was equal to 10 nM. These results indicate that TNF-α / SPD-304 interactions are probably more stable in the absence of DMSO, resulting in a greater affinity between the considered entities.

5. Conclusion

In the present study, we demonstrate that surface acoustic wave devices can be used to quantify the affinity between TNF-α protein and SPD-304 ligand in one hand, and to monitor their kinetic of binding in the other hand. In the majority of studies involving this type of system, DMSO cosolvent is used systematically to solubilize compounds in aqueous buffers. Here, we showed that it is possible to avoid its systematic employ. In fact, when only HBS-E buffer is used; the biosensor presents a limit of detection as low as 10 nM, and the dissociation constant value is one order of magnitude lower than that found in presence of DMSO. The non-use of DMSO cosovent in the reaction medium enhances probably the affinity between the involved species.

References

[1] H. Wajant, K. Pfizenmaier, P. Scheurich, Tumor necrosis factor signaling, Cell Death Differ. 10 (2003) 45-65.
[2] R. Alzani, A. Cortin, L. Grazioli, E. Cozzi, P. Ghezzi, Suramin induces deoligomerization of human tumor necrosis factor alpha. J. Biol. Chem. 268 (1993) 526 – 12529.
[3] F. Mancini, C. M. Toro, M. Mabilia, M. Giannangeli, M. Pinza M, C. Milanese C, Inhibition of tumor necrosis factor-α (TNF-α) / TNF-α receptor binding by structural analogues of suramin, Biochem. Pharmacol, 58 (1999) 851-859.
[4] M M. He, A. S. Smith, J. D.Oslob, W. M. Flanagan, A. C Braisted, A. Whitty, M. T. Cancilla, J. Wang, A. A. Lugovskoy, J. C. Yoburn, A.D. Fung, G. Farrington, J. K. Eldredge, E. S. Day, L. A. Cruz, T. G. Cachero, S. K. Miller, J. E. Friedman, I. C. Choong, B. C. Cunningham., Small-molecule inhibition of TNF-alpha, Science. 310 (2005) 1022-1025.
[5] N. Bracke, S. Barhdadi, E. Wynendaele, B. Gevaert, M. D'Hondt, B. De Spiegeleer, Surface acoustic wave biosensor as a functional quality method inpharmaceutics, Sens. Actuators B. 210 (2015) 103-112.
[6] L. Sangdae, K. Yong-Il, K. Ki-Bok, Comparative study of binding constants from love wave surface acoustic wave and surface plasmon resonance biosensors using kinetic analysis, J. Nanosci. Nanotech, 13 (2013) 7319-7324.
[7] T. de F. Paulo, H. Abruna, I. Diogenes, Thermodynamic, Kinetic, Surface pKa, and Structural Aspects of Self-Assembled Monolayers of Thio Compounds on Gold, Langmuir, 28 (2012) 17825-17831.
[8] N. Fourati, M. Seydou, C. Zerrouki, A. Singh, S. Samanta, F. Maurel, D. K. Aswal, M. Chehimi, Ultrasensitive and Selective Detection of Dopamine Using Cobalt-Phthalocyanine Nanopillar-Based Surface Acoustic Wave Sensor, ACS Applied Materials & Interfaces 6 (2014) 22378-22386.
[9] T. M.A. Gronewold, Surface acoustic wave sensors in the bioanalytical field: Recent trends and challenges, Anal. Chim. Acta 603(2007) 119-128.
[10] Y. Bergaoui, C. Zerrouki, N. Fourati, J.M. Fougnion, A. Abdelghani, Antigen-antibody selective recognition using $LiTaO_3$ SH-SAW sensors: investigations on macromolecules effects on binding kinetic constants. Eur. Phys. J. Appl. Phys. 56 (2011) 13705.
[11] D. H. Tsai, S. Elzey, F. W. Delrio, A. M. Keene, K. M. Tyner, J. D. Clogston, R. I. Maccuspie, S. Guha, M.R. Zachariah, V. A. Hackley, Tumor necrosis factor interaction with gold nanoparticles, Nanoscale, 4 (2012) 3208–3217.
[12] C. P. Papaneophytou, A. K. Mettou, V. Rinotas, E. Douni, G. A. Kontopidis, Solvent Selection for Insoluble Ligands, a Challenge for Biological Assay Development: A TNF-α/SPD304 Study, ACS Med. Chem. Lett, 4 (2013) 137−141.