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Abstract

$ ODEHO IUHF UHDO WLPH PHWKRG ZDV GHYHORSHG IRU WKH GHWHFWLRP¡ ZHDC ¨+ DQG FKDQJH LQ HQWURS\ ¨6 ZHUH GHWHUPLQHG DQG WKH YDOXHV UHYHDO and BoNT/A antibody as spontaneous, endothermic and entropy driven one. In order to optimize the detection method, temperature and pH variation studies were also performed.

Keywords: Biological warfare agent; SPR; Impedance; BoNT/A

Introduction

e use of hazardous materials from chemical or biological produced mainly by ve Clostridium botulinum types A through G (BoNT/A-G)

) and translocation ( ) domains. Crystal structure analysis of the BoNT/A reveals that the H ( ) clearly consists two distinct subdomains [4] that is N-terminal domain ( ) and C-terminal domain ( ) and are speculated to bind with a protein receptor and gangliosides [5-8]. e H fragment in its isolated

e most associated serotypes with human food-borne infection are

Numerous analytical methods are developed to detect BoNTs including various biological assays such as mouse bioassay, enzym linked immunosorbent assay and surface plasmon resonance based assay. Mouse bioassay is a highly sensitive assay that directly measur the amount of functional toxic BoNTs. However, it has a well-known limitation that it usually requires 24 h to 10 days to conduct experiment and requires animals. Various SPR assays were reported for the measurement of binding of BoNT/A to ganglioside GT1b [12], BoNT/A to monoclonal antibodies (MAbs) [13] including synaptic vesicles chips assay [14] and protein chip membrane capture assay [15]. A number of assays for BoNTs have been developed, aiming to equal the sensitivity of the mouse lethality assay while improving its limitation [16-25]. ough a lot of methods have been reported for BoNTs detection still better method development and improvisation are needed.

Protein chips technologies are promising one in chemical biology for detection and quanti cation of proteins and can be automated, multiplexed and miniaturized [26,27]. Among the di erent protein chip methodologies, SPR allows for real-time and label-free detection of molecular interactions between immobilized ligand on a sensor chip and analytes injected over the surface [28,15].

In continuation to our earlier studies on the development of detection methodologies for biological and chemical warfare agents [29-31], in the present work we have developed a label-free real-time SPR optical method for the sensing of BoNT/A with carboxymethyldextran modi ed gold chip (CM5 chip). Interaction of BoNT/A with immobilized antibody was conducted. Moreover, interaction of

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concentration dependant angle changes, upon the interaction of various concentrations of BoNT/A with immobilized antibody and the Figure 2A depicts the corresponding calibration curve. e limit of detection (LOD) of the present method was calculated experimentally and was found to be 0.045 fM and this is the minimum concentration of BoNT/A antigen which showed the response during the interaction of BoNT/A antigen with its immobilized antibody.

Binding of di erent concentration of BoNT/A antigen was also carried out with immobilized SV protein in order to know the biological activity of puri ed protein and the sensorgrams are shown in Figure 2B. Figure 3 represents the comparative response of binding of BoNT/A and BSA with immobilized SV. BSA was used as negative control and showed 63% binding with immobilized SV in comparison of BoNT/A as shown in Figure 3.

Evaluation of kinetics involved in the antigen and antibody interaction

e a nity interactions between immobilized BoNT/A antibody and BoNT/A antigen were characterized by the equilibrium constant (K_D). e data were tted using a simple 1:1 interaction model [37], A + B = AB, where 'A' is the injected analyte, 'B' is the immobilized ligand and 'AB' is the analyte–ligand complex formed during the interaction process. In the SPR system, the signal R is proportional to the amount of (AB) and the Rmax is proportional to the initial (B). Hence, in this study kinetic parameters such as K_D and Bmax value were calculated

Evaluation of thermodynamic parameters

e K_D obtained from kinetic evaluation so ware was further exploited to calculate the thermodynamic parameters such as change in Gibb's free energy (G), change in enthalpy (H) and change in entropy (S) associated with binding of BoNT/A antigen with immobilized antibody using Van't Ho [39,40] equation:

$$\Delta G \quad - \qquad _A = \Delta H \; - \; T\Delta S \tag{1}$$

$$\Delta H \qquad \frac{T\,T}{T\;-T} \quad \frac{K}{K} \tag{2}$$

$$K_A = \frac{}{K_D} \tag{3}$$

where, R: universal gas constant, K_A: ka nity constant, T: temperature and K_1, K_2 are a nity constants for association at T_1 and T_2 temperature, respectively. G, H and S are change in Gibb's free energy, change in the enthalpy and change in the entropy due to the binding of BoNT/A with immobilized BoNT/A antibody, respectively.

e value of the change in Gibb's free energy for binding of BoNT/A with immobilized BoNT/A antibody, was found to be -84.99 kJ/mol at 298 K. e negative value of G indicates the spontaneous interaction of BoNT/A antigen with its antibody.

e calculated value of H using Von't Ho Wizard in kinetic evaluation so ware was found to be +16.33 kcal/mol. e positive value of H reveals the interaction of BoNT/A with immobilized BoNT/A antibody as endothermic [41].

e value of S for binding of BoNT/A with immobilized BoNT/A antibody was calculated using so ware and was found to be 207.43 cal $mole^{-1}$ K^{-1}. e magnitude of T S value was found to be higher than H indicating that the net in uence of enthalpy on the BoNT/A with immobilized BoNT/A antibody is minor and the apparent gain in entropy is actually the driving force for the BoNT/A with immobilized BoNT/A antibody [42,43]. e observed positive value in entropy indicated that the interaction can be explained by Langmuir replacement reaction that exhibits Langmuir type isotherm [38]. Langmuir replacement reaction suggests that the observed positive entropy is because of desorption of water molecules from either antibody or antigen or both. e signi cance of desorption of water molecules from protein surfaces during ligand-protein binding was reported earlier [43].

E ect of temperature

Temperature variation study was performed in order to know the e ect of temperature on SPR response during the interaction of antibody with antigen. Upon increasing temperature from 10 to 25°C an increase in SPR angle is observed as shown in Figure 4A and beyond 25°C, SPR angle decreased. Hence, 25°C is used as optimum temperature for the interaction of BoNT/A antigen with its immobilized antibody.

E ect of pH

Figure 4B shows the e ect of pH on SPR angle due to the interaction of BoNT/A antigen with its immobilized antibody. It is observed from Figure 4B that SPR response exhibits a maximum at pH 7.5. is result can also be explained by considering the e ecn 5e de expgy. Id anect Ints(t).1(b)1(t). .6(b)t. Iivr. IP(a)12(ue0(r. I)31d)13(esur. I)

parameter to re ect the in-situ changes those are occurring on the modi ed disc/electrolyte interface, hence, t and simulation method was adopted to nd out the Ret value for CM5 gold disc and antibody immobilized CM5 gold chip before and a er antigen interaction, the Ret values are found to be 2.79 M , 466 k and 440 k , respectively. e decrease in Ret value a er the interaction of antigen with antibody directly con rms an increase in electron transfer due to the e ective binding of antigen with antibody because of the presence of good interaction at this pH (7.5) as reported earlier for an antigen antibody interaction [44].

Conclusion

A label free real time SPR detection methodology for BoNT/A was developed using a carboxymethyldextran modi ed sensor chip. e e ective interaction between the antigen and antibody was con rmed based on EIS data as a decrease in charge transfer resistance was observed due to the e ective interaction between BoNT/A antigen and antibody. e LOD of the developed method is 0.045 fM. e SPR sensor gram of the binding of BoNT/A with immobilized SV con rmed the biological activity of puri ed protein. e kinetic parameters such as k_a and Bmax values were calculated and found to be 0.53 fM and 38.23°n for immobilized antibody and 0.22 fM and 116.0 n for immobilized SV, respectively. e K_D val n-3(i)12(t)20.53 fM implies and classi es this antibody as a high a nity one. e negative value thange in Gibbs free energy indicates the spontaneous nature otteraction of BoNT/A antigen with immobilized antibody and SV and the positive value tevealed the interaction of BoNT/A with immobilized antibody as endothermic. e positive val n-3(i)12(t)2T S was found to be greater than2 H indicating the interaction of BoNT/A with its immobilized antibody as entropy driven. is study gives inputs for the development of SPR based sensors using antigen and antibody interaction for BoNT/A and other BWAs.

References

1. Sakaguchi G (1982) Clostridium botulinum toxins. Pharmacol 19: 165-194.
2. Hambleton P (1992) Clostridium botulinum toxins: a general review of involvement in disease, structure, mode of action and preparation for clinical use. J Neurol 239: 16-20.
3. Turton K, Chaddock JA, Acharya KR (2002) Botulinum and tetanus neurotoxtins: structure, function and therapeutic utility. Trends Biochem Sci 27: 552-558.
4. Lacy DB, Tepp W, Cohen AC, Gupta BRD, Stevens RC (1998) Crystal structure of botulinum neurotoxin type A and implications for toxicity. Nat Struct Biol 5: 898-902.
5. Kitamura M, Takamiya K, Aizawa S, Furukawa K (1999) Gangliosides are the binding substances in neural cells for tetanus and botulinum toxins in mice. Biochim Biophys Acta 1441: 1-3.
6. Kozaki S, Kamata Y, Watarai S, Nishiki T, Mochida S (1998) Ganglioside GT1b as a complementary receptor component for Clostridium botulinum neurotoxins. Microb Pathog 25: 91-99.
7. Li L, Singh BR (1998) Isolation of synaptotagmin as a receptor for types A and E botulinum neurotoxin and analysis of their comparative binding using a new microtiter plate assay. J Nat Toxins 7: 215-226.
8. Ginalski K, Venclovas C, Lesyng B, Fidelis K (2000) Structure-based sequence alignment for the beta trefoil subdomain of the clostridial neurotoxin family provides residue level information about the putative ganglioside binding site. FEBS Lett 482: 119-124.
9. Arnon SS, Schechter R, Inglesby TV, Henderson DA, Bartlett JG, et al. (2001) Botulinum toxin as a biological weapon: medical and public health management. JAMA 285: 1059-1070.
10. Bigalke H, Rummel A (2005) Medical aspects of toxin weapons. Toxicology 214: 210-220.
11. Johnson EA (2003) Bacterial Pathogens and Toxins in Foodborne Disease. In Food Safety: Contaminants and Toxins pp: 25-45.
12. Yowler BC, Schengrund CL (2004) Botulinum neurotoxin A conformation upon binding to ganglioside GT1b. Biochemistry 43: 9725-9731.
13. Pless DD, Torres ER, Reinke EK, Bavari 6 +LJK DI¿QLW\ SU antibodies to the binding domain of botulinum neurotoxin type A, Infection and Immunity 69: 570-574.
14. Ferracci G, Miquelis R, Kozaki S, Seagar M, Leveque C (2005) Synaptic vesicles chips to assay botulinum neurotoxins. J Biochem 391: 659-666.
15. Marconi S, Ferracci G, Berthomieu M, Kozaki S, Miquelis R, et al. (2008) A protein chip membrane capture assay for botulinum neurotoxin activity. Toxicology and Applied Pharmacology 233: 439-446.
16. Doellgast GJ, Triscott MX, Beard GA, Bottoms JD, Cheng T, et al. (1993) Sensitive enzyme-linked immunosorbent assay for detection of Clostridium ERWXOLQXP QHXURWR[LQV $ % DQG (XVLQJ VLJQ coagulation assay. J Clin Microbiol 3: 2402-2409.
17. Ekong TA, Mc Lellan K, Sesardic D (1995) Immunological detection of Clostridium botulinum toxin type A in therapeutic preparations. J Immunol Methods 180: 181-191.
18. Szilagyi M, Rivera VR, Neal D, Merrill GA, Poli MA (2000) Development of sensitive colorimetric capture ELISAs for Clostridium botulinum neurotoxin serotypes A and B. Toxicon 38: 381-389.
19. Schmidt JJ, Stafford RG, Millard CB, (2001) High throughput assays for botulinum neurotoxin proteolytic activity: serotypes A, B, D, and F, Anal. Biochem 296 130-137.
20. Chiao DJ, Shyu RH, Hu CS, Chiang HY, Tang SS (2004) Colloidal gold-based immunochromatographic assay for detection of botulinum neurotoxin type B. J Chromatogr B Analyt Technol Biomed Life Sci 809: 37-41.
21. %DUU -5 0RXUD + %R\HU $(:RRO¿WW $5 .DOE 6 neurotoxin detection and differentiation by mass spectrometry Emerg Infect Dis 11: 1578-1583.
22. Gessler F, Hampe K, Bohnel H (2005) Sensitive detection of botulinum QHXURWR[LQ W\SHV & DQG ' ZLWK DQ LPPXQRDI¿QL Appl Environ Microbiol 71: 7897-7903.
23. Sapsford KE, Taitt CR, Loo N, Ligler FS (2005) Biosensor detection of botulinum toxoid A and staphylococcal enterotoxin B in food. Appl Environ Microbiol 71: 5590-5992.
24. Sharma SK, Eblen BS, Bull RL, Burr DH, Whiting RC (2005) Evaluation of ODWHUDO ÀRZ &ORVWULGLXP ERWXOLQXP QHXURW Appl Environ Microbiol 71: 3935-3941.
25.

detection. Anal Biochem 353: 248-256.

26. Cherif B, Roget A, Villiers CL, Calemczuk R, Leroy V, Marche PN, Livache T, Villiers MB (2006) Clinically related protein–peptide interactions monitored in