Journal of Medical Virology 86:363–371 (2014)

Characterization of Pandemic Influenza A (H1N1) Virus Hemagglutinin Specific Polyclonal Antibodies for Biosensor Applications

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In this study, recombinant hemagglutinin protein (rH1N1HA) of Pandemic influenza virus and polyclonal antibodies against it for biosensor applications have been characterized. For rapid and high sensitive detection of H1N1 virus or its antibodies, PCR-free and label free detection method based on a surface plasmon resonance technique has been proposed. The glycosylated H1N1HA protein was expressed in yeast and the authenticity of the expressed protein was confirmed by Western blotting. Rabbit polyclonal antibodies developed against rH1N1HA protein were evaluated for their ability to neutralize H1N1 virus through plaque reduction neutralization test and indirect ELISA. Affinity purified anti-H1N1HA IgG were characterized further for their specificity, affinity of interaction, the association and dissociation rates at which they interact through surface plasmon resonance technique. The equilibrium constant and maximum binding capacity of analyte was found to be 49.7 nM and 47.28m°, respectively. The assay could detect a lowest IgG of 0.5 ng on a rH1N1HA coated chip. Combined with the high sensitivity of surface plasmon resonance technique and specificity of the reagents, it is possible to develop a rapid detection assay for monitoring influenza infections. ***J. Med. Virol. 86:363–371, 2014***.

KEY WORDS: pandemic influenza A (H1N1) virus; biosensor; biomolecular interaction; hemagglutinin; surface plasmon resonance

INTRODUCTION

Pandemic influenza is caused by influenza A virus that belongs to the family Orthomyxoviridae. Influenza A viruses are characterized by subtype by the two major surface glycoproteins, haemagglutinin, and neuraminidase. Rapid detection of pandemic influenza at national or regional level is a public health issue of critical importance. Extensive mortality and morbidity have been associated with the pandemics of influenza outbreaks in the past, thus indicating the need to develop a rapid and sensitive assay for early warning of the infection. There is an urgent need for rapid and reliable detection methods for monitoring human viral pathogens, such as H1N1 influenza. For rapid and highly sensitive detection of clinically relevant viruses, methods other than PCR have been proposed and developed based on biosensing applications [Kim et al., 2010; Driskell et al., 2011]. Biosensors are used widely in research and industrial fields, such as early diagnosis of diseases, medicinal analysis, detection of toxic substances in foods, and warfare agent detection [Song et al., 2008]. There are several techniques such as enzyme-linked immunosorbent assay (ELISA), Western blotting, MALDI, mass spectrometry [Claudia et al., 2008] for identifying suitable biosensors for diagnostic applications. The surface plasmon resonance (SPR) technique is accepted widely for studying biomolecular interactions in real-time with a high degree of sensitivity and without the need of label. SPR has often been used for the characterization of antibodies [Johne et al., 1993; Polymenidou et al., 2005] and probably is closest to being the reference method for studying antibody–antigen interactions [Regenmortel et al., 1998]. The information obtained from SPR is both qualitative and quantitative and it is possible to obtain the kinetics of the interaction. High sensitivity of the SPR effect to the refractive index changes of the near surface region, for example, induced by trapped biomolecules has made the SPR

Grant sponsor: DRDE, Gwalior, India

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Accepted 26 July 2013

DOI 10.1002/jmv.23753

Published online 23 September 2013 in Wiley Online Library (wileyonlinelibrary.com).

effect especially attractive for pharmacology research. SPR based detection methods have been previously reported based on aptamers for detecting avian influenza [Hua et al., 2012]. Similarly an SPR assay has been reported for detecting Influenza virus using the HA proteins of H1N1 and H3N2 [Nilsson et al., 2009]. A paired surface plasma waves biosensor (PSPWB) has been reported recently for rapid and sensitive detection of swine-origin influenza A H1N1 virus [Su et al., 2012]. Considering the practical advantages, the SPR method of detection is one of the most often investigated procedures used for detection of biomolecules smaller than 200 nm in diameter that have been immobilized at different surfaces. This new technology has been used in the present study to examine the interaction kinetics of pandemic influenza (H1N1) hemagglutinin and its corresponding purified immunoglobulin G (IgG). The principal interest of this study is to define the characteristics of recombinant H1N1 virus Hemagglutinin protein and its antibody in terms of their specificity of interaction; the association and dissociation rates at which they interact and their affinity, how tightly they bind to each other.

MATERIALS AND METHODS

Molecular Reagents, Pichia Strain and Plasmid

DNA polymerase, restriction enzymes, T4 DNA ligase were purchased from Fermentas (Glen Burnie, MD). Synthetic oligonucleotides and chemicals were purchased from Sigma–Aldrich (Bangalore, India). *Pichia pastoris* GS115 (Invitrogen, Carlsbad, CA) was used as the host strain for expression of the Pandemic H1N1 virus Hemagglutinin protein. The yeast transfer vector pPIC9K was from Invitrogen.

Expression and Purification of rH1N1HA Protein in Yeast (*P. pastoris*)

The synthetic gene of the Hemagglutinin from novel California/04/2009 H1N1 was cloned into pPICK9K yeast transfer vector (Invitrogen) at *Eco*RI and *Not*I sites in frame with α-mating factor secretion signal under the control of the methanol-inducible *P. pastoris* alcohol oxidase 1 (AOX1) promoter. Recombinant Pichia cells (GS115–H1N1HA) of His^+, Mut^+ genotypes were generated by recombining the *Sal*I linearized pPIC9KH1N1HA with *P. pastoris GS115* cells via electroporation as previously described by us [Athmaram et al., 2011; Athmaram et al., 2012]. Positive transformant (GS115–H1N1HA) having multi-copies of the HA expression cassette was methanol induced to express the H1N1 HA protein [Athmaram et al., 2012]. The yeast expressed protein was purified via DEAE Sepharose anion exchange chromatography using Acta explorer (GE Healthcare, San Diego, CA) as described by us earlier [Athmaram et al., 2013]. The purified protein was analyzed on SDS–PAGE gel and the protein was visualized after Coomassie staining. The authenticity of the expressed protein was confirmed by Western blotting using commercial antibodies as described earlier [Athmaram et al., 2013]. The concentration of H1N1 HA was determined using Bradford assay [Bradford, 1976].

Development of Polyclonal Antibodies Against Purified rH1N1HA Protein in Rabbits

Four healthy adult male New Zealand White rabbits that were seronegative for H1N1 were immunized intramuscularly with 50 μg each of yeast derived HA protein in combination with Freund's complete adjuvant (FCA) (Sigma Chemicals, St. Louis, MO). For negative control, one rabbit was similarly immunized with PBS-adjuvant. After 2 weeks interval, animals were boosted twice with the same amount of protein in combination with Freund's incomplete adjuvant (FIA) (Sigma Chemicals). Following 2 weeks of the second booster dose, blood samples were collected from marginal veins and the sera were separated. The reactivity of the rabbit hyperimmune sera against cell culture grown purified H1N1 virus was determined via Indirect ELISA and virus neutralization assay. The animal study experiments had an approval from the Institutional Animal Ethics Committee (IAEC) and Institutional Biosafety committee (IBSC) as per the institutional norms.

Virus Neutralization Assay

The heat inactivated sera samples collected from immunized rabbits were subjected to in vitro virus neutralization assay at different dilutions (1:32, 1:64, 1:128) using pandemic H1N1 virus isolated from Bangalore, India as reported earlier by us [Athmaram et al., 2011]. The negative control of the experiment consisted the virus diluted in nonimmunized rabbit serum. Pre-incubated serum/virus mixtures were layered on MDCK cells grown in six-well plates and incubation was continued for 1 hr at room temperature. After the adsorption of virus–serum mixture, the inoculum was aspirated out from the wells and overlaid with 1.5% low melting point agarose (Sigma Chemicals). Plates were incubated for plaque formation at 37°C, 5% CO_2 for 3 days and the wells were stained with 0.2% crystal violet (in 30% ethyl alcohol) solution. The plaques were counted and the neutralization activity of the immune sera was assessed by comparing the plaque numbers obtained from that of negative control serum. The highest dilution of the sera that showed more than 50% reduction in plaque number than that of negative control is considered as the neutralizing titer.

Indirect ELISA to Demonstrate the Reactivity of Anti-HA HyperImmune Sera

Ninety-six well Maxisorp plates (NUNC) were coated with purified inactive H1N1 virus (100 ng/well in 0.5 M sodium carbonate–bicarbonate buffer, pH 9.6) by incubating at 4°C overnight. The plate

was then washed three times briefly with PBS-T and the wells were blocked using 3% BSA dissolved in PBS-T (1 hr at 37°C). The wells were then washed three times briefly with PBS-T. The rabbit anti-HA sera from all four HA immunized animals along with negative control (PBS administered) were diluted serially (10^{-2} to 10^{-5} in PBS-T plus 1% BSA) and 100μl of the sera was added to each well in duplicates in a predetermined order and incubated for 1 hr at 37°C. After incubation the wells were washed briefly (five times, 3 min each) with PBS-T and incubated with anti-rabbit horse radish peroxidase-conjugated secondary antibody (Sigma–Aldrich) diluted 1:5,000 in PBS-T for 1 hr at 37°C. Finally, the wells were washed with PBS-T (five times, 3 min each). Color development was done by incubating the wells with 100μl each of tetramethyl benzidine/H_2O_2 liquid chromogen-substrate solution (Sigma–Aldrich, St. Louis, MO) for 5 min. The reactions were stopped by adding 2 M sulphuric acid (50μl/well) and the absorbance was measured at 450 nm.

Affinity Purification of Immunoglobulin G (IgG)

The IgG were purified from the pooled sera collected from the rH1N1HA immunized rabbits using Protein A affinity 5 ml column (GE Healthcare) using AKTA explorer as per the methods described by the manufacturer. Briefly Protein A Sepharose column was equilibrated in Phosphate buffered saline, pH 7.4 and the H1N1HA specific IgG present in the serum were loaded onto the affinity column in the same buffer at a flow rate of 1 ml/min. The nonspecific proteins were washed in the same buffer and the bound IgG were eluted in 0.1 M glycine buffer, pH 3.0 at a flow rate of 1 ml/min. The collected fractions of 1 ml volume were neutralized immediately with 1 M Tris–HCl at pH 9.0. The peak corresponding to IgG was collected and quantified using Bradford assay [Bradford, 1976].

Surface Plasmon Resonance Analysis

Chemicals, reagents and instruments. 4-Mercaptobenzoic acid (4-MBA), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), phosphate buffered saline (PBS), sodium acetate, ethanolamine, and hydrochloric acid (HCl) were obtained from Fluka. Glacial acetic acid, glycine, and NaOH were supplied by Sigma–Aldrich chemie, Steinheim, Germany (San Diego, CA). All chemicals and reagents used were of analytical grade and purification was performed wherever necessary before use.

The biomolecular interactions were investigated using a two channel cuvette based electrochemical surface plasmon resonance system (Autolab ESPRIT, Ecochemie, Utrecht, The Netherlands) and here Gallium-Arsenide (Ga-As) diode laser is a source with a fixed wavelength of 670 nm in conjunction with a scanning mirror to modulate the plane polarized light beam on the SPR substrate. The outcome of the SPR measurement was monitored automatically using a PC with data acquisition software version 4.3.1. All kinetic data were obtained using kinetic evaluation software version 5.0 (Ecochemie B.V, The Netherlands). Bare SPR gold chip was modified with 4-MBA by using spin coater (Autolab ESPRIT, Ecochemie B.V., The Netherlands). The pH of the buffers was measured with a EUTECH instruments pH meter (pH-1500, Eutech instruments Pvt. Ltd, Ayer Rajah Cresent, Singapore). All experiments were carried out at 25°C unless otherwise specified and the temperature of cuvette was controlled by a Julabo HE-4 (Julabo Labortechnik, Seelbach, Germany) water bath.

Immobilization of Antigen on 4-MBA Modified Gold SPR Sensor Chip

Prior to the immobilization of rH1N1HA antigen (Ag) on 4-MBA modified gold chip, 50 μl of PBS buffer (pH 7.4) was passed every 120 sec interval for 600 sec in order to get a stable baseline in both the channel. 4-MBA modified gold chip was chemically activated by the injection of 50 μl of a 1:1 mixture of 400 mM EDC and 100 mM NHS. Subsequently, 50 μl of antigen (1:500 dilution in 10 mM PBS) was injected in channel 1 for 1,800 sec to get an effective immobilization of rH1N1HA antigen on the activated 4-MBA modified gold chip surface. Following antigen immobilization, the remaining active sites were then blocked with 50 μL of 1000 mM ethanolamine. Afterwards, 10 mM HCl was injected to achieve regeneration of immobilized sensor surface. For negative control measurements, the modified gold surface was activated with EDC/NHS and then quenched with ethanolamine in channel 2 as mentioned above and was used as blank control surface.

Bio-Sensing Protocol

For the binding (biosensing) studies of antibody, different dilutions of anti-rH1N1HA polyclonal antibodies were prepared in PBS. The entire SPR sensing methodology was executed by a sequence of automatic procedure (baseline, association, dissociation, regeneration, and back to baseline steps). A sample solution containing a selected concentration of anti-rH1N1HA polyclonal antibodies in the PBS was injected in both channels from the 384-well microtiter plate and then association was performed for 500 sec and dissociation was performed for 400 sec followed by the regeneration of the sensor surface by addition of 10 mM HCl for 120 sec. PBS (pH 7.5) was used as the running buffer solution throughout the study. For SPR sensing, an aliquot of the analyte solution (75 μl) was injected and mixed at 16.7 μl sec^{-1} and this protocol was adopted with (a) 1:6,400, (b)

1:1,600, (c) 1:400, (d) 1:100, (e) 1:50 dilution of anti-rH1N1 HA polyclonal antibodies.

RESULTS

H1N1 HA Expression and Purification of rec.H1N1HA Protein Via Anion Exchange Chromatography

The GS115–H1N1HA Pichia transformant after 72 hr of methanol induction expressed recombinant HA protein (~80 kDa) into the culture supernatant. Uninduced negative control did not show such band (data not shown). The recombinant HA protein was purified using DEAE Sepharose anion exchange chromatography column. The bound HA protein on the exchange column was recovered using 200 mM NaCl as shown in Figure 1. The 20 mM Tris–Cl, pH 7.72 buffer combined with a linear gradient of 1 M NaCl at a flow rate of 1 ml/min was found to be good in purifying the rec.HA protein. On SDS–PAGE gel, the fractions corresponding to the protein peak eluted using gradient NaCl showed single band that matched the size of H1N1 HA protein as seen in Figure 2A. The concentration of the purified protein as estimated by Bradford assay was 280μg/ml. The purified rec.HA protein was analyzed for its authenticity via immunoblotting using commercial anti-H1N1 HA specific antibodies (GenScript USA Inc. Piscataway, NJ). As seen in Figure 2B, the purified rH1N1HA protein was recognized by the anti-H1N1 HA antibodies.

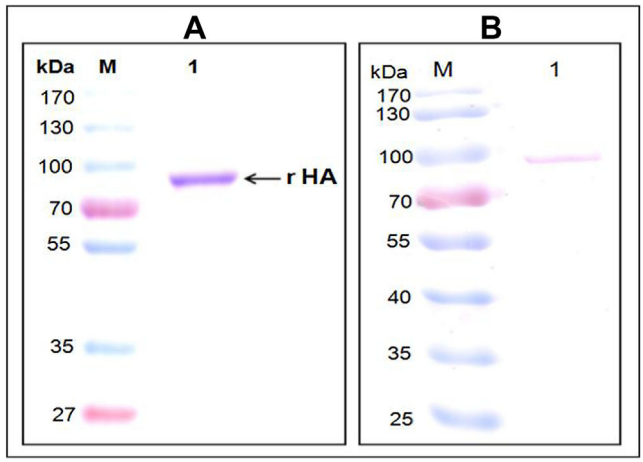

Fig. 2. **A**: SDS–PAGE analysis of anion exchange chromatography purified rH1N1HA protein. Lane M: Pre stained molecular marker (Fermentas, Cat. no.: 1811), Lane 1: rH1N1HA fraction eluted with from linear NaCl gradient (110–120 ml fraction). **B**: Western blot analysis of purified rH1N1HA protein: Lane M: Prestained molecular marker (Fermentas, Cat. no.: SM0671), Lane 1: Anion exchange chromatography purified rH1N1HA. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jmv]

In Vitro Neutralisation Activity of H1N1HA Immunized Rabbit Sera (Plaque Reduction Neutralization Test)

Plaque Reduction Neutralization Test (PRNT) was performed on the immunized rabbit sera collected

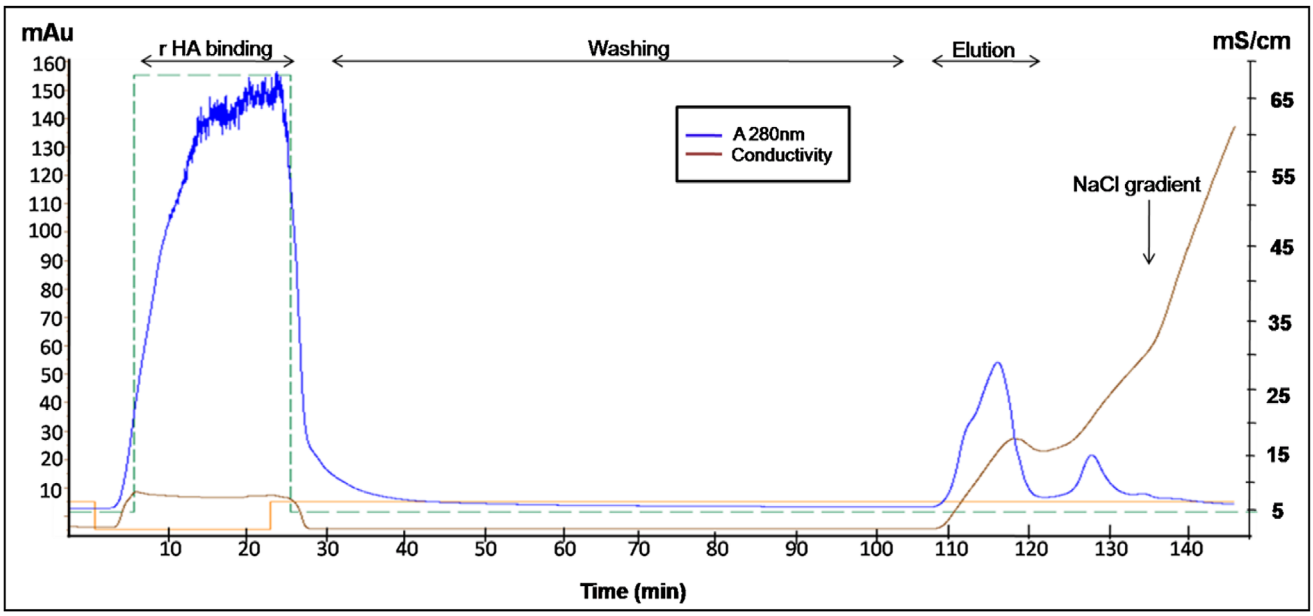

Fig. 1. Anion exchange chromatogram of rH1N1 HA purification. The crude HA protein diluted in equilibration/binding buffer (20 mM Tris–Cl, pH 7.72) was loaded onto DEAE -Sepharose anion exchange column and nonspecific proteins were washed with 20 mM Tris–Cl, pH 7.72. The bound protein was eluted with sodium chloride linear gradient (0–1 M NaCl in 20 mM Tris–Cl, pH 7.72) at a flow rate of 1 ml/min using Akta explorer (GE Healthcare). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jmv]

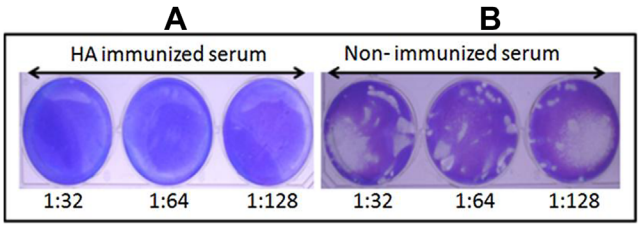

Fig. 3. Virus neutralization activity of rabbit hyperimmune serum raised against yeast derived rH1N1HA protein. Different dilutions (1:32, 1:64, and 1:128) of immunized (panel A) and nonimmunized (panel B) rabbit sera were incubated with H1N1 virus and the resulting serum-virus mixture was infected onto cultured MDCK cells. The plates were observed for reduction in the plaque numbers after incubation to determine the presence or absence of virus neutralizing antibodies in the hyperimmune serum. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jmv]

after 2 weeks of second booster. The twofold diluted sera (1:32, 1:64, and 1:128) that were challenged with 100 PFU of H1N1 virus showed 50% reduction in plaque numbers in all dilutions tested (Fig. 3, panel A). Whereas, the sera from the control nonimmunized rabbit did not show any significant reduction in the plaque numbers at any of the dilutions tested (Fig. 3, panel B).

Indirect ELISA

Indirect ELISA of rH1N1HA immunized sera revealed the presence of H1N1HA reacting antibodies (Fig. 4). No significant reactivity was noticed in case of sera from rabbit that was immunized with PBS-adjuvant control. The antibody titer varied from animal to animal and from Figure 4, it is evident that the hyperimmune sera diluted even up to 10^{-5} was able to recognize the H1N1 virus. Thus the ELISA method employed in the present study reconfirmed the elicitation of H1N1 virus specific IgG in the immunized rabbits.

Purification of Anti-H1N1HA IgG Via Affinity Chromatography

Upon affinity purification, the main peak of IgG, was exclusively isolated from affinity Protein A Sepharose column using 0.1 M glycine containing elution buffer at pH 3.0, and the remaining nonspecific proteins were removed with 100 mM Tris–Cl buffer, pH 7.2 (Fig. 5). The obtained IgG yield as determined by Bradford method was 5.10 mg/ml.

Surface Plasmon Resonance Analysis

Immobilization of rH1N1HA antigen on 4-MBA modified SPR sensor chip. Figure 6 represents the stepwise immobilization of rH1N1HA antigen on 4-MBA modified gold chip and this process comprises of nine steps as reported earlier [Gupta et al., 2012]. As seen in Figure 6, in first step, stabilization of baseline was performed for 120 sec. For chemical binding between the 4-MBA adsorbed on the gold surface and free amino groups of rH1N1HA antigen, in second step, activation of carboxyl groups on 4-MBA modified chip was carried out for 900 sec with EDC-NHS. In third step, washing was conducted with PBS and the SPR angle shifted nearly to baseline [Tsai and Li, 2009]. In fourth step, rH1N1HA antigen was injected on 4-MBA modified gold chip, allowed to interact 1,800 sec and an

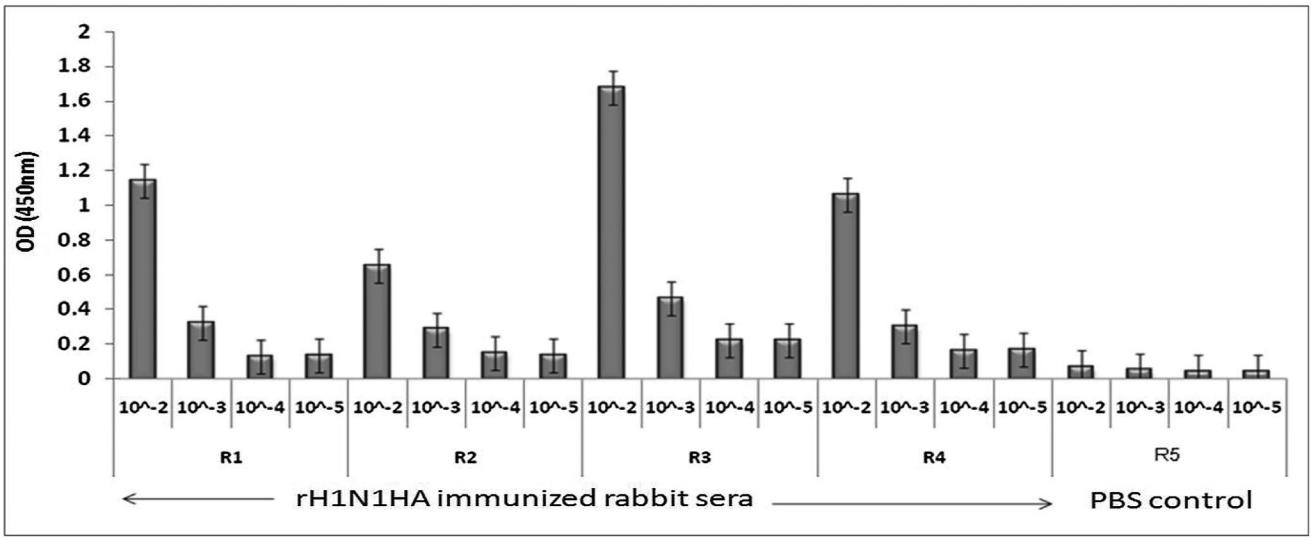

Fig. 4. Indirect ELISA to demonstrate the reactivity of anti-HA hyperimmune sera. Reactivity of the rabbit anti-HA sera collected from four HA immunize rabbits (R1–R4) and PBS administered negative control (R5) at different dilutions (10^{-2} to 10^{-5}) against inactivated H1N1 virus coated on microtiter plate wells. Optical density was measured after enzymatic color development that confirms the presence of H1N1 virus specific IgG in the immunized rabbit sera samples.

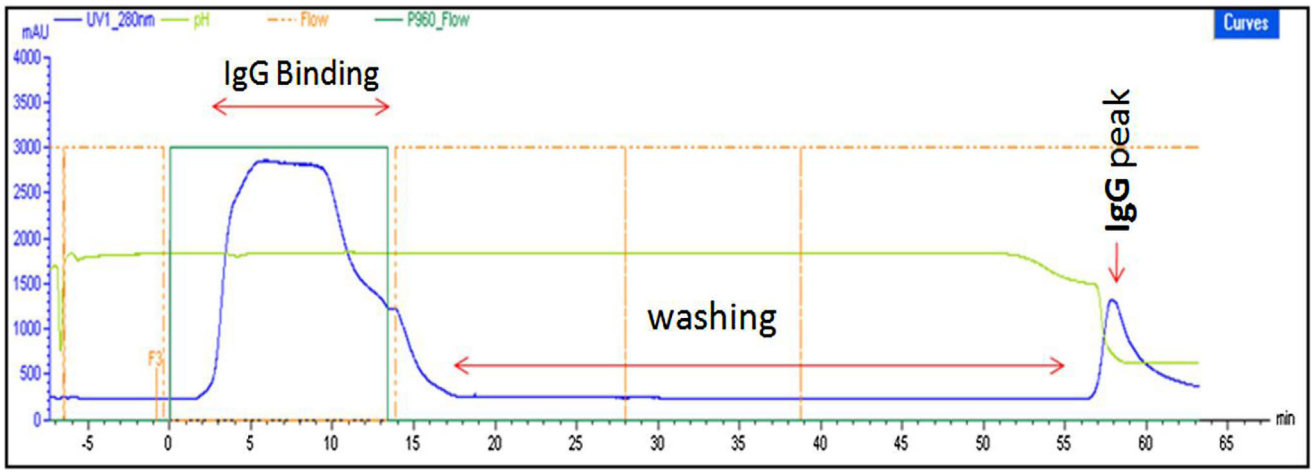

Fig. 5. Protein A affinity purification of anti-H1N1 IgG using Akta explorer. The rabbit hyperimmune serum against yeast derived rH1N1HA protein was loaded onto the affinity column to bind IgG. The unbound proteins were washed in buffer containing 100 mM Tris–Cl (pH 7.2) and finally the specifically bound IgG were eluted in buffer containing 0.1 M glycine (pH 3.0). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jmv]

increase in SPR angle is observed. In fifth step, washing was performed and in sixth step, to prevent nonspecific binding and also for the blocking of unreacted NHS-ester groups on 4-MBA chip, 1,000 mM ethanolamine was used and allowed to react with sensor surface for 600 sec. In seventh step, washing was performed for 30 sec as discussed in the experimental part. In eighth step, regeneration was carried out for 120 sec. At last step, back to base line process was conducted for 60 sec. From Figure 6, a net angle change of 332.25m° is observed and this ascribes the attachment of 2.77 ng/mm^2 of rH1N1HA antigen on 4-MBA modified gold chip [Stenberg et al., 1991].

Interaction of Anti-rH1N1HA Polyclonal Antibodies With the Immobilized rH1N1HA Antigen on 4-MBA Chip

The rH1N1HA antigen immobilized sensor chip was utilized for the sensing of different concentrations of anti-rH1N1HA polyclonal antibodies and the

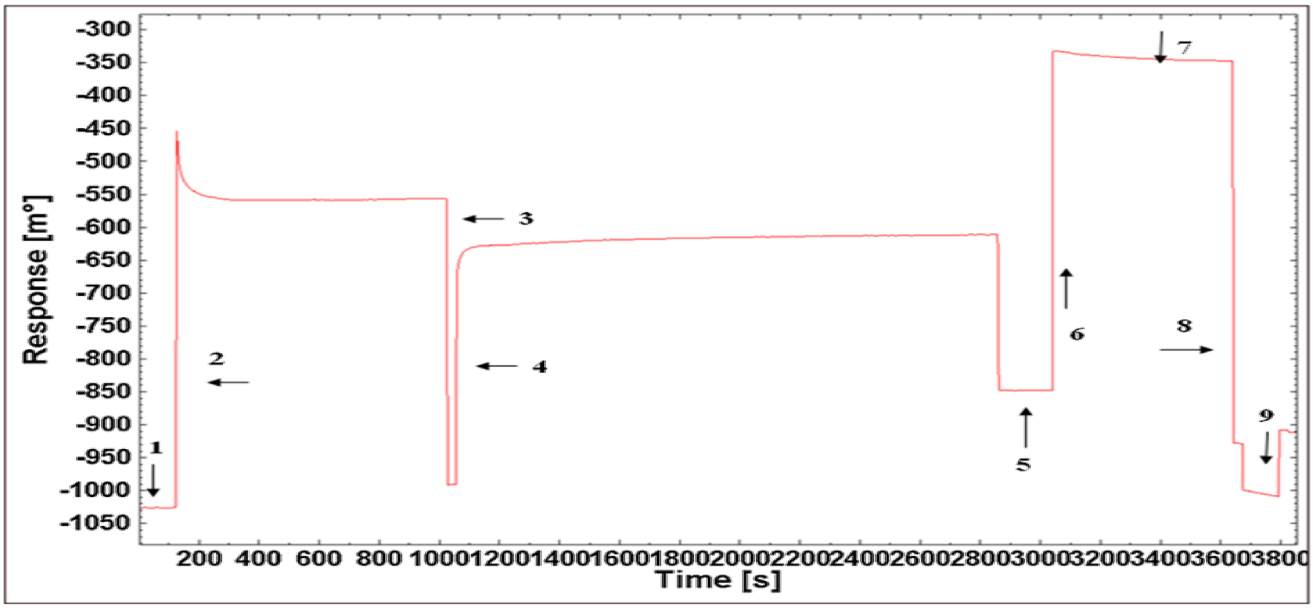

Fig. 6. Sensorgram showing different steps: involved in the immobilization of rH1N1HA Ag (1:500 dilution) on 4-MBA modified gold chip. (1) baseline, (2) EDC-NHS activation, (3) washing, (4) antigen coupling, (5) washing, (6) deactivation, (7) washing, (8) regeneration, and (9) back to baseline. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jmv]

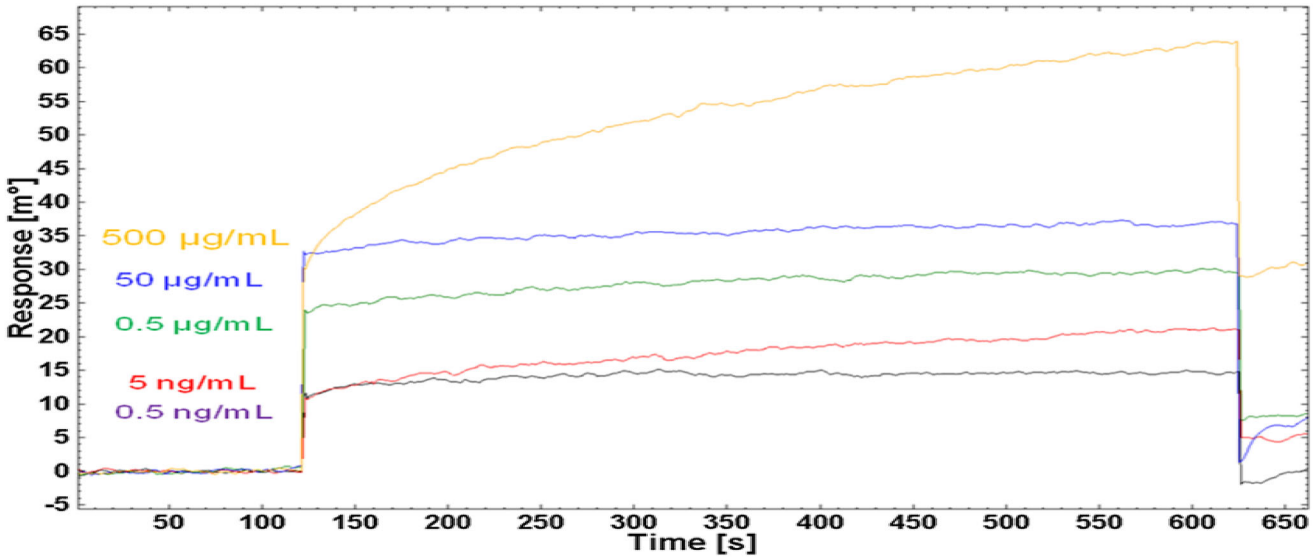

Fig. 7. SPR sensor response for the interaction of different dilution of rH1N1HA polyclonal antibodies with immobilized recombinant H1N1HA antigen. (a) 1:6,400, (b) 1:1,600, (c) 1:400, (d) 1:100, (e) 1:50. Temperature: 25°C and pH: 7.5.

net results are depicted as SPR sensorgram in Figure 7 and this sensorgram exhibits a concentration dependant angle changes, upon the interaction of various concentrations of anti-rH1N1HA polyclonal antibodies with its immobilized Ag. A calibration curve was plotted using the data of Figure 7 and the resultant figure is presented as Figure 8 which exhibits a regression coefficient of 0.99. The limit of detection (LOD) of the present method was calculated experimentally and was found to be 0.5 ng/ml of rH1N1HA Ag during the interaction with its immobilized anti-rH1N1HA polyclonal antibodies on 4-MBA chip.

Evaluation of Kinetics Involved in Between the Interaction of Anti-rH1N1HA Polyclonal Antibodies With Immobilized Recombinant H1N1HA Antigen

The affinity interactions between immobilized antigen and antibody were characterized by the equilibrium constant (K_D). Kinetic parameters such as K_D

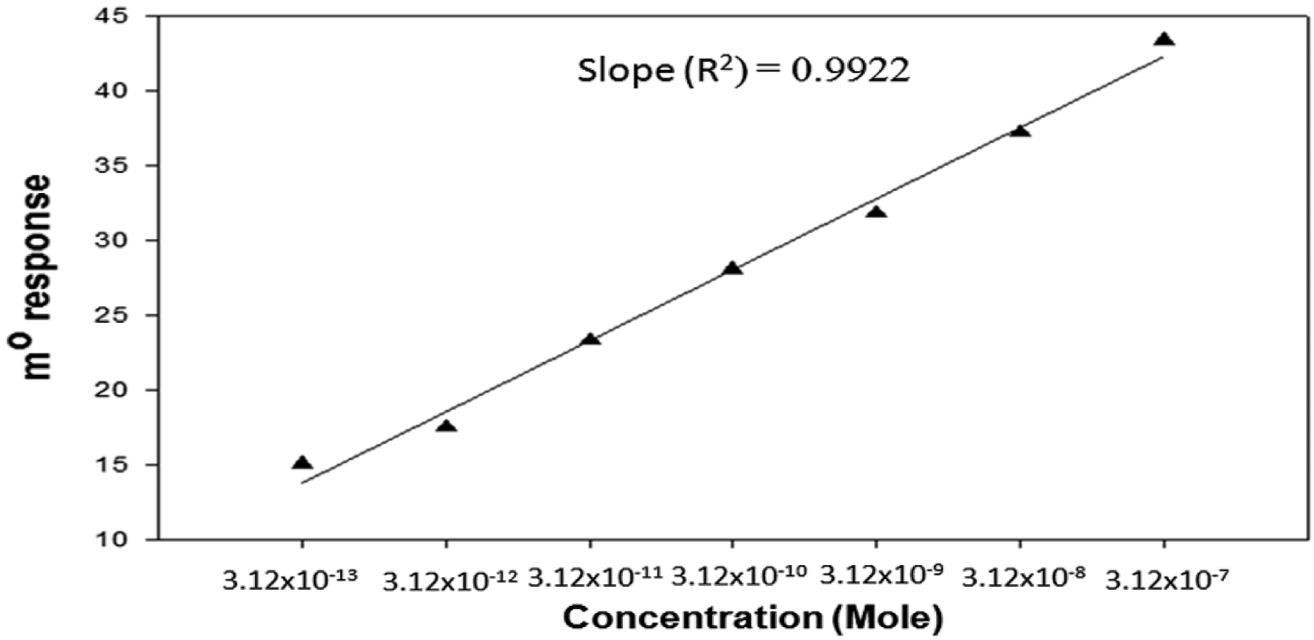

Fig. 8. Linearity curve showing the interaction of anti-rH1N1HA IgG concentration versus response time.

and B_{max} value were calculated for binding of anti-rH1N1HA polyclonal antibodies with immobilized recombinant H1N1 HA antigen using the software and were found to be 49.7 nM and 47.28m°, respectively.

Evaluation of Thermodynamic Parameters

The Gibb's free energy for binding of anti-rH1N1HA polyclonal antibodies with immobilized Ag was found to be −41.60 kJ/mol at 298 K. The calculated value of ΔH using Von't Hoff Wizard in kinetic evaluation software was found to be −49.2 kcal/mol. The value of ΔS for binding of antibody with immobilized Ag was found to be −127.78 cal/mol K.

DISCUSSION

The expression of recombinant H1N1HA is in agreement with that of our previous publication [Athmaram et al., 2012]. The observed in vitro neutralization activity of recombinant H1N1HA protein se results are also in agreement with that of previous reports by [Steitz et al., 2010] where neutralization titers ≥640 were observed in mice after HA immunization. Similar titer of 1:256 in mice [Athmaram et al., 2011] and PRNT titer of 1:160 in ferrets [Wang et al., 2011], were observed. The observed low PRNT titer could be attributed to the difference in animal species. The in vitro neutralization data further strengthens the presence of H1N1 virus specific neutralizing antibodies in the rabbit hyperimmune sera developed against rH1N1HA protein. The ELISA test in the present study reconfirmed the elicitation of H1N1 virus specific IgG in the immunized rabbits. The IgG affinity purification based on *Staphylococcus aureus* protein A is widely practiced as this protein can efficiently trap Fc region of IgG from rabbit sera [Lindmark et al., 1983; Hermanson et al., 1992]. Most of the non-IgG proteins were eliminated in the flow of starting Tris–Cl buffer at pH 7.2. However in the present study, further separation of IgG fractions to the subclasses was not attempted on Protein A Sepharose column with stepwise elution gradient of pH as it was not required for subsequent experiment. Affinity chromatography with Protein A Sepharose at pH 3 was shown to be a powerful method for the isolation and purification of IgG antibodies from rabbit serum. This method is attractive since a mild condition is used and, furthermore, since several of the proteins normally found in serum do not bind to the affinity column. The capacity of the immunoaffinity column is thereby used for binding the protein of interest. Generally, immunoaffinity column with protein A appears to be particularly suited for the isolation of IgG molecules from serum.

In order to understand the interaction of anti-H1N1HA antibodies with the immobilized antigen on the SPR chip, the K_D value was used further to calculate thermodynamic parameters such as change in Gibb's free energy (ΔG), change in enthalpy (ΔH), and change in entropy (ΔS) using Van't Hoff equation [Glasstone, 1947; Savara et al., 2009]. The negative value of ΔG obtained in the present study indicates the spontaneous interaction of antibody with the antigen. The calculated value of ΔH (−49.2 kcal/mol) reveals the interaction of antibody with immobilized Ag as exothermic process [Maenaka et al., 2001]. The ΔS value (−127.78 cal/mol K) obtained in the present study indicates the strong binding of antibody with immobilized Ag. The magnitude of TΔS value was found to be higher than ΔH indicating that the net influence of enthalpy on the antibody with immobilized antigen is minor and the apparent gain in entropy is actually the driving force for the antibody with immobilized Ag [Cabilio et al., 2000]. The thermodynamic parameters deduced indicate that the interaction between the antibody and immobilized antigen on SPR chip is exothermic, entropy driven and spontaneous process. In general, the characterized rH1N1HA antigen and the corresponding antibodies in the present study have immense application both in detection of H1N1 specific antibodies and the virus respectively. Combined with the high sensitivity of the Surface Plasmon resonance technique and specificity of reagents, it is possible to develop a rapid detection assay for monitoring Influenza infections in near future. However the present observations are confined to the recombinant H1N1 antigen and virus specific antibodies. The SPR assay has to be evaluated with large number of clinical samples that was beyond the scope of this study as it requires a High containment facility to handle and test the H1N1 clinical samples.

CONCLUSION

The present study proposes the development and characterization of highly sensitive and specific biomolecules for rapid detection of H1N1 virus without the need for PCR amplification. The obtained results reveal that HA specific antibodies can find potential applications in SPR based detection methods for rapid and accurate diagnosis of H1N1 viral infection via appropriate pathogen-specific antibody in the near future. There is a great potential to fabricate cost-effective label free SPR biosensors for rapid detection of H1N1 influenza virus or antibodies against H1N1 infection that helps in monitoring pandemic Influenza.

ACKNOWLEDGMENTS

The authors are thankful to Dr. B.K. Bhattacharya, Head, Bio-process scale up facility for his support. The authors also acknowledge Dr. Beer Singh, Head, Protective Device Division for his help on SPR analysis. The authors deeply acknowledge Prof. (Dr) M. P. Kaushik, Director, DRDE, Gwalior, Madhya

Pradesh 474 002 for his support in executing this study.

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