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ORIGINAL ARTICLE

Real Time and Label-Free Research on the Detection of Pituitary Adenylate Cyclase-Activating Polypeptide Based on Surface Plasmon Resonance Technique

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SUMMARY

Background: **The self-developed portable surface plasmon resonance (SPR) biosensor was used in the quantitative detection and kinetic study of pituitary adenylate cyclase-activating polypeptide (PACAP), as the existing detection methods were complicated, with a long detection period, high-cost instrument, and sample needing to be labeled.**

Methods: **After preparing SPR biochip, the direct detection proceeded in an immune reaction detection between the PACAP samples with concentrations of 0.5 mg/L, 1 mg/L, 2 mg/L, 5 mg/L, 8 mg/L, 10 mg/L, and PACAP type 1 receptor (PAC1R). The standard curve of PACAP direct detection was established. According to the 1:1 Langumair model, the immune responses dynamic characteristic parameters of PAC1R with PACAP-38, and their reconstructive PN37R, PK38W were calculated, respectively.**

Results: **The direct detection limit of PACAP could be 0.5 mg/L. The absolute deviation and relative deviation of the detected value and the true value are both low. The magnitude orders of kinetic parameters of immune response between PAC1R and PACAP-38, PK38W, PN37R are basically the same. However, the specific values of the binding rate constant and dissociation rate constant of PK38W and PN37R are slightly larger than that of PACAP-38.**

Conclusions: **The experimental results show that the SPR biochip detection system can be used for the effective quantitative detection of PACAP and can be used for kinetic study. The developed device could provide a label-free, simple, quick, and low-cost method for the concentration detection of PACAP in the samples and the immune response study between PACAP and its receptors. It could promote PACAP to play an important role in the pharmaceutical industry, clinical treatment, and other industries.**

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INTRODUCTION

Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is a kind of neuropeptide capable of activating adenylate cyclase activity in cultured rat pituitary cells which was originally isolated from the sheep hypothalamus [1,2]. PACAP has two active forms as PACAP-38 and PACAP-27 [3]. PACAP could play a role in the biological functions of neurotransmitters/ regulators, neurotrophic factors, and immune system regulation when it is in a different organization or system. For example, by activating PACAP type 1 receptor (PAC1R) through the pituitary adenylate cyclase, PACAP could reduce the damage to neurons, promote the survival of neurons, and play a role in the nutritional or protective effects on neurons [4,5] during early embryonic development or in the mature brain. PACAP modulates the immune function of dendritic cells (DC) that are activated by lipopolysaccharide (LPS), which can improve the occurrence and development of infectious diseases and rheumatism [6,7], and thus plays a therapeutic role. Therefore, the concentration detection of PACAP in the biological fluids and the immune response study between PACAP and its receptors could promote PACAP to play an important role in the pharmaceutical industry, clinical treatment, and other industries. The traditional methods of PACAP detection are radioimmunoassay (RIA), indirect immunofluorescent antibody (IFA) method, and enzyme linked immunosorbent assay (ELISA). It is difficult to meet the requirements of rapid detection as the detection limits of those methods are low, while the instruments are expensive and the sample processing steps are complex with long detection periods and complex operations. More importantly, samples usually need to be labeled; the biological activity of the sample may be destroyed, and the fluorescent tag will also pollute the environment [8-10]. Therefore, it is necessary to establish a label-free, rapid, sensitive, simple, and low-cost method for PACAP detection.

Surface plasmon resonance (SPR) biosensors combine the high specificity of the immune response with the high sensitivity of surface plasmon resonance photoelectric detection high sensitivity, and there is no need for labeling and special detection equipment with strong specificity, simple operation, simple sample pretreatment. Therefore, SPR biosensors have been widely used in many fields, such as biology, medicine, chemistry, drug screening, and environmental monitoring [11-13]. Because of the superior performance of SPR biosensor detection technology, some commercial instruments have been developed [14], for example Biacore AB produced BIAcore series which is currently the most mature product line, but they are expensive and bulky (as the price of BIAcore 2000 is more than $200000, and its volume is 760 mm x 350 mm x 610 mm, with net weight of 50 kg). Small SPR instruments, such as Spreeta2000 of Texas Instruments and SensiQ of Nomadics, are limited in measuring range or sensitivity or ease of operation; some can only be used as special instruments for detecting a small number of samples.

The self-developed angle scanning portable SPR biosensor was used in this study to investigate the quantitative detection method of PACAP-38 and the dynamic reaction process of PACAP and PAC1R by the specificity of immune response. Compared to the traditional PACAP detection method, the developed instrument is portable, with easy operation, label-free, low cost, and can be used for rapid on-site detection. The method is expected to be used in PACAP related clinical treatment and basic pharmaceutical fields.

MATERIALS AND METHODS

Experimental equipment

Principles of immune detection for SPR technology

The SPR effect is a kind of physical optical phenomenon occurring on the interface between the dielectric layer and the metal layer [15-19]. The SPR effect is very sensitive to the refractive index change of the dielectric on the surface of the noble metal film, and refractive index is the inherent characteristic of all materials. Therefore, the biochemical analysis based on the SPR effect does not need to label the sample, and the biochemical analysis of the sample can be realized by detecting the small change of the refractive index of the sample [20-24]. For the biochemical analysis using SPR, the modified biochip is fixed with a biological probe (antigen or antibody), when the sample containing matched receptors flows through the chip surface, the analyte (receptor) will be captured on the surface of the chip because of the affinity reaction, causing the increase of the refractive index, and then the resonance state of the reflected light will change accordingly. By adding buffer solution containing or not containing the substance to be detected, the process of analyte binding and dissociation could be monitored in real-time. With the change of reaction time (abscissa), the refractive index of the sample under test changes which causes the change of resonance wavelength or resonance angle (ordinate). Therefore, the interaction between biomolecules can be detected by the relationship between the incident light wavelength or angle and the liquid refractive index on the surface of the metal film.

Figure 1 is the schematic diagram of the immune response test results of the SPR detecting instrument [23]. After the biochip was successfully prepared, the buffer was injected into the chip surface to record the SPR response at this time and act as the baseline for detection; samples containing different concentrations of analyte are injected into the chip surface, and the analyte is bound to the biological probe immobilized on the chip surface; the higher the analyte concentration, the faster the binding rate, and the faster the refractive index change, which reflects the rapid increase of SPR response value. After the analyte adsorbed on the chip is saturated, the buffer was injected into the chip, and the

analyte will dissociate from the chip surface. After the regeneration solution is injected, the chip is regenerated, and the response value is returned to the detection baseline, and then the next sample can be detected continually.

Through the detection of the immune reaction, the binding kinetic process of antigen-antibody can be analyzed, and furthermore, the characteristic parameters of antigen-antibody reaction can be calculated to describe the reaction rule of antigen-antibody. The dynamic analysis using SPR should be combined with the actual chemical or biological significance to analyze the sensor data. When the reaction mode of the analysis system is not clear, the Langumair model should be used first. According to the Langumair model [25], the 1:1 type reaction occurring on the sensor chip can be expressed as follows:

$$dR/dt = -k_d\ R \quad (1)$$

$$dR/dt = k_a\ C_A\ (R_{max} - R) - k_d\ R$$
$$= k_a\ C_A\ R_{max} - k_a\ C_A\ R - k_d\ R \quad (2)$$
$$= k_a\ C_A\ R_{max} - (k_a\ C_A + k_d)\ R$$

where, C_A is the concentration of the analyte; R_{max} is the resultant response for forming the most complex on the sensor chip; R is the response signal at time t, in the SPR system established in this paper, the response signal is the sample resonance angle; k_a is the association rate constant; k_d is the dissociation rate constant.

Experimental SPR device

As shown in Figure 2A, the self-developed portable scanning SPR biosensor including SPR biosensor chip, optical system, circuit system, circulation system, and software platform. Angle scanning is performed with a voltage controlled high precision galvanometer, and the reflected light intensity is detected by a photocell; the method is simple, with a portable device (both optical and circuit systems are integrated into a unified mechanical framework, with a size of 550 mm x 200 mm x 330 mm); the circulation system uses a linear motor driven micro injector instead of a peristaltic pump, which can avoid the pulse flow generated during the operation of a peristaltic pump and ensures the accuracy of injection.

Figure 2B is the optical path in the SPR system schematic. The incident light is guaranteed in a horizontal direction, the zero point of the galvanometer is 45° to the horizontal direction, the initial position of the mirror and the horizontal direction is at an angle of 37°, and the initial incident angle of the cylindrical mirror is guaranteed to be 74° (at room temperature, the resonance angle of distilled water is about 74°, and the resonance angle of most samples is near that of the distilled water). When the galvanometer counter has a clockwise deflection angle of θ, and the incidence angle of the cylindrical mirror has a deflection of 2θ, that is to say the incidence angle of cylindrical mirror is 74° - 2θ. On the contrary, when the galvanometer has a clockwise deflection angle of θ, the incidence angle of the cylindrical mirror is 74° + 2θ. The SPR resonance angle of the sample can be calculated by looking for the corresponding mirror angle when the sample SPR curve appears at resonance peak. The measuring range of the system is mainly decided by the galvanometer scanning angle range and prism parameters. According to the relation between prism and sample refractive index:

$$n_2^2 = \varepsilon\ (n_p)^2\ (\sin\theta)^2 / [\varepsilon - (n_p)^2\ (\sin\theta)^2] \quad (3)$$

where, n_2 is the refractive index; ε is the metal dielectric constant; n_p is the refractive index of the prism; θ is the incidence angle of the prism. The prism is K9 glass in the system, then ε = -11, n_p = 1.5163; in order to ensure that the spot is in the center of the prism, the measured range of the galvanometer scanning angle can reach to ± 5°, the incidence angle θ of the gold film is 64° - 84° at that time, the corresponding refractive index range is 1.2415 - 1.3762, as shown in Figure 3. This range is wide enough to measure most biochemical solution samples. If the range of refractive index measurement needs to be further improved, a prism with larger refractive index should be chosen.

The measurement accuracy of the system mainly depends on the measurement accuracy of the angle scanning system and the photoelectric detection device. The angle scanning system is a voltage controlled galvanometer, its angle resolution is up to 0.001°, the resolution angle of the corresponding prism is 0.002°. The conversion accuracy of photoelectric detector to light intensity is 0.001 V, the resolution of data acquisition card (16 bit ADC) can reach 7.7 x 10^{-5} V, that is enough to distinguish the light intensity change of the angle of 0.002°. Therefore, the accuracy of the whole system can reach 0.002°. According to formula (3), in the scanning range of the system, the differential of refractive index n_2 was calculated when dθ = 0.002° was used as the interval, the corresponding refractive index measurement resolution ratio of angle resolving accuracy is about 1.8 x 10^{-5} - 4.2 x 10^{-6} in the range of 64° - 84°. In order to improve the precision of the SPR sensor and anti-interference ability, the statistical average filter, low-pass filtering, data fitting, and other methods in the aspect of data processing were applied to suppress noise interference in addition to optimization in hardware. According to the test, direct detection for glucose solution can reach 0.1 mg/mL or even lower [26]. After repeated measurement for the same analyte (distilled water), the relative standard deviation RSD ≈ 0.0014%, that is to say the stability and reproducibility of the system is good. When kinetic tests were performed with mouse antigen and antibodies, we can get the association rate constant $k_A \approx 2.05 \times 10^4$ M-s- and dissociation rate constant $k_d \approx 0.005$ s-. As shown in reference [27], the association rate constant and dissociation rate constant of rabbit anti human IgG and human IgG tested by BIAcore X Biosensor System were $k_a \approx 9.37 \times 10^4$ M-s- and $k_d \approx 0.00383$ s-, respectively; The results of the same type of antigen and antibodies are consistent in magnitude with what we obtained with the established system. The SPR system designed in this paper has the advantages of ingeniously designed light path, stable

Table 1. Analysis of detection results of PN37R concentration.

Prepared concentration	Detected concentration (First time)	Detected concentration (Second time)	Detected concentration (Third time)	Mean concentration	Absolute deviation	Relative deviation	Standard deviation
3.00 mg/L	2.92 mg/L	2.95 mg/L	2.93 mg/L	2.93 mg/L	0.070	0.023	0.015
4.00 mg/L	3.98 mg/L	3.99 mg/L	4.01 mg/L	3.99 mg/L	0.010	0.002	0.015
6.00 mg/L	6.01 mg/L	6.01 mg/L	6.02 mg/L	6.01 mg/L	0.010	0.001	0.006

Table 2. Calculation of kinetic parameters.

Kinetic parameters	PN37R	PACAP-38	PK38W
k_a	3.01 x 10^7 M-s-	1.47 x 10^7 M-s-	4.34 x 10^7 M-s-
k_d	1.09 x 10^{-2} s-	5.32 x 10^{-3} s-	1.65 x 10^{-2} s-

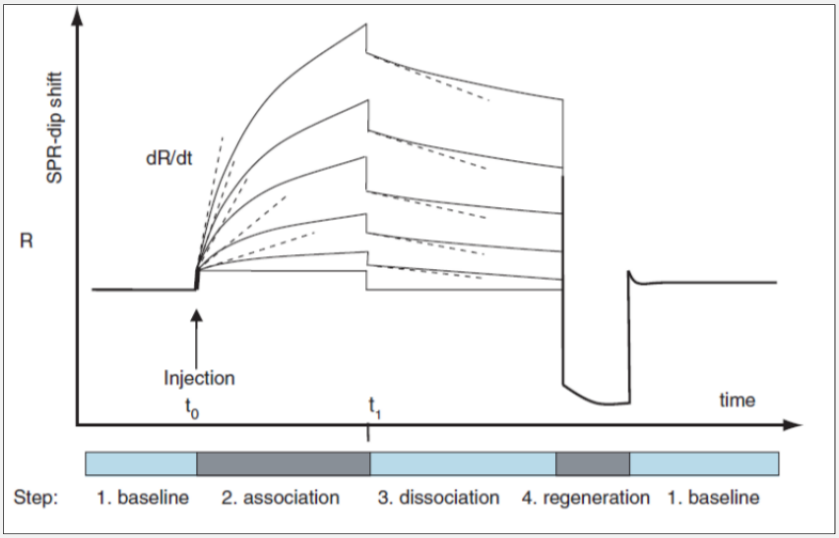

Figure 1. Schematic of SPR sensor immune detection process.

and portable machine, with low cost and large range of refractive index detection.

Chemicals and reagents

In this study, the pituitary adenylate cyclase activating peptide samples were donated by the Key Laboratory of Bioengineering Drugs of Jinan University (type 1 receptor was an extracellular fragment, with molecular weight of about 42 kDa; ligands including PACAP-38 and modified PK38W, PN37R, PACAP-38 have a molecular weight of about 4297.01 Da, PK38W has a molecular weight of about 4593.34 Da, and PN37R molecular weight of about 4577.39 Da); thioglodecanoic acid [$HS(CH_2)_{10}COOH$], mercaptohexanoic acid [$HS(CH_2)_6$-OH] Ethylamine, Ethyl Dodecyl Sulfate (N), N-Hydroxysuccinimide (NHS), [N-ethyl-N'-(Dimaminopropyl) carbodiimide, EDC] were bought from Sigma, USA; other reagents were purchased from Beijing Chemical reagents company. PBS was used as the buffer for the immunoreaction (2 mmol/L NaH_2PO_4, 2 mmol/L Na_2-HPO_4, 150 mmol/L NaCl, pH7.4).

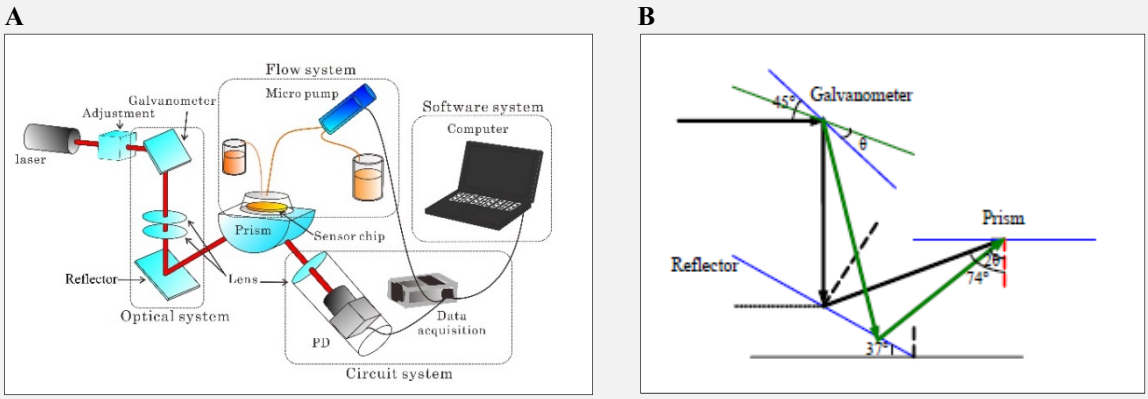

Figure 2. (A) Structure diagram of the SPR system; (B) Diagram of optical path.

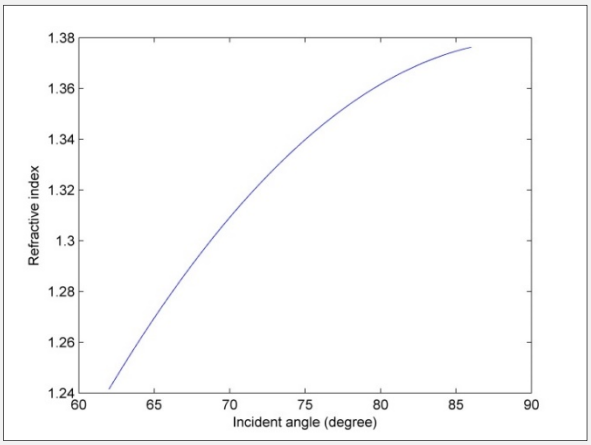

Figure 3. The curve of incidence angle and the refractive index.

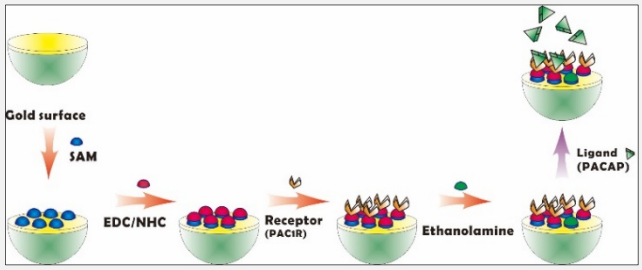

Figure 4. Diagram of PACAP detection process by SPR.

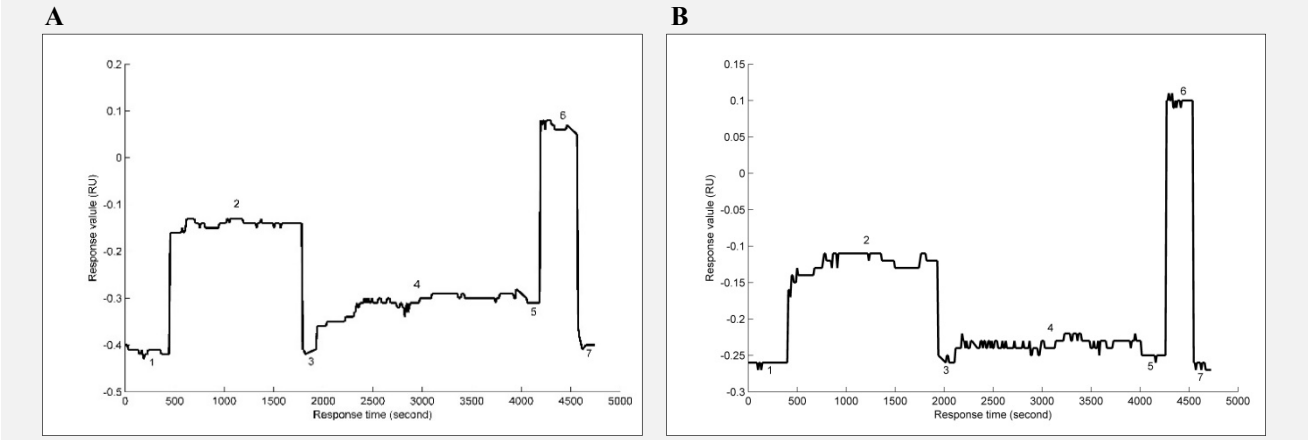

Figure 5. (A) Recording curve of biological chip preparation; (B) Recording curve of failure in biological chip preparation.

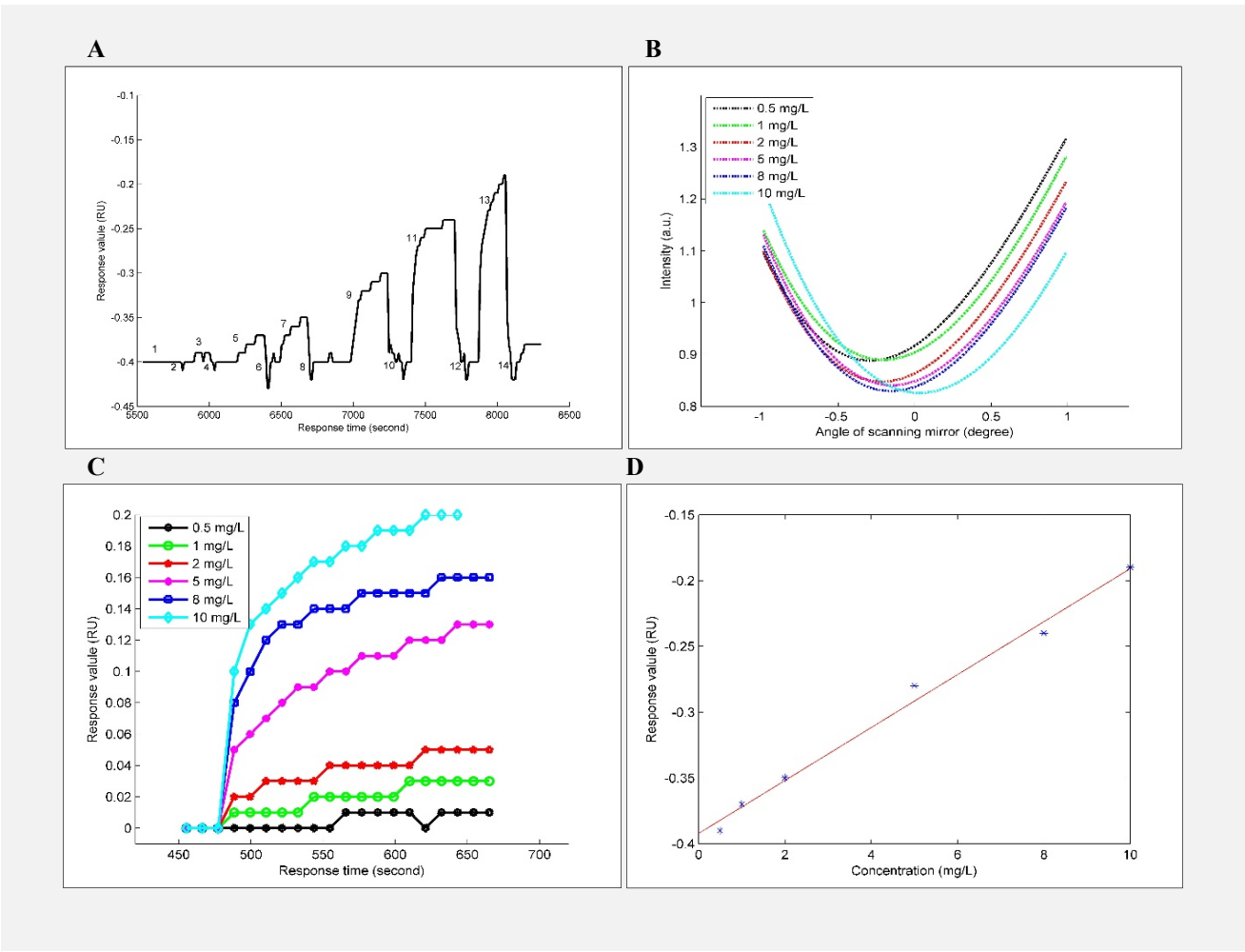

Figure 6. (A) Recording curve of direct detection of PN37R; (B) SPR curve for direct detection of different concentrations of PN37R; (C) Kinetic curves of direct detection of PN37R; (D) Standard curve of direct detection of PN37R.

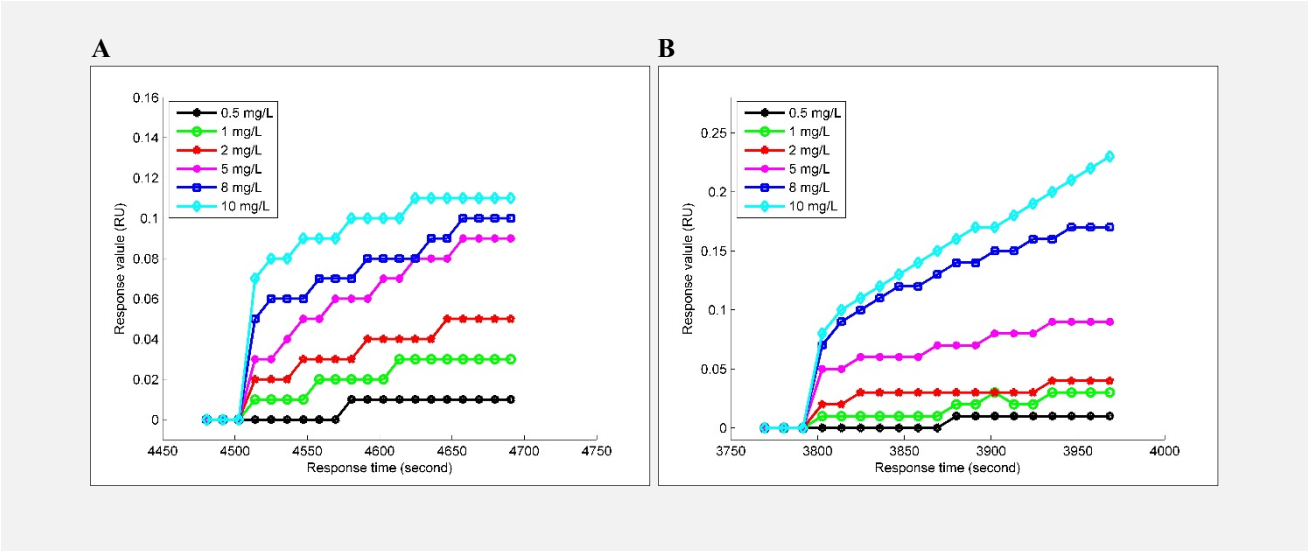

Figure 7. (A) Kinetic curves of direct detection of PACAP-38; (B) Kinetic curves of direct detection of PK38W.

EXPERIMENT AND DISCUSSION

Preparation of biochip

Figure 4 shows the immunoassay process of PACAP by the SPR optical method, including biochip modification, chip activation, bioprobe immobilization, inactivation, and immunoreaction. The process of modification, activation, fixation and inactivation was the preparation process of the biochip. The specific operational method is as follows: the circular glass plate with diameter of 20 mm and the thickness of 1 mm is chosen as the base of the biosensor chip, and gold with thickness of 50 nm is deposited on it. Then, the gold film is prepared by self-assembled monolayer (SAM) of ethanol solution of $HS(CH_2)_{10}COOH$ (mercapto-undecanoic acid) and $HS(CH_2)_6OH$ (mercaptohexanoic acid), with 1 mmol/L and mass ratio (M/M) of 1:9; self-assembly modification for the surface of the gold film was lasted for 2 - 2.5 hour, mercapto and gold would bond in the modified liquid, and result in the formation of carboxyl. The refractive index of cedar oil (refractive index of 1.51 - 1.52) is similar to that of the cylindrical prism (K9 glass) used in the experiment. The biosensor chip with gold film is placed on the prism of the SPR instrument with the cedar oil used as a coupling agent. The circulation system is installed, the PBS buffer is entered, and the SPR scanning parameters are set: scan starting point -1°, scan range 2°, scan step 0.01°. Reducing the scanning step can further improve the system resolution, but will increase the time of a single scan and reduce the measured data points. Therefore, it should be set according to the actual needs of the scanning parameters. PBS buffer (2 mmol/L NaH_2PO_4, 2 mmol/L Na_2HPO_4, 150 mmol/L NaCl, pH7.4) was injected onto the surface of the chip, and the SPR response was started to record using PBS's resonance angle as baseline (stage 1 of Figure 5A, the vertical axis is the time; the horizontal axis is the change of the resonance angle of the SPR, measured by the reaction unit RU). After the stabilization of baseline, the mixture of 0.1 mol/L NHS and 0.1 mol/L EDC (1:1, V/V) was injected to activate the surface of the chip for about 20 minutes in order to activate the carboxyl group on the surface of the chip into an active ester, where the SPR response value is increased (stage 2 of Figure 5A); after rinsing with PBS for 2 minutes (step 3 of Figure 5A), PAC1R was fixed on the surface of the biochip as a biological probe at a concentration of 300 ppm. The SPR response value at that point was significantly higher (stage 4 of Figure 5A). After about 40 minutes and rinsing with PBS for 2 minutes, the SPR response value is slightly reduced at that point (stage 5 of Figure 5A), indicating that the probe fixation is good; 1 mol/L ethanolamine (pH 8.5) was added to inactivate the remaining ester bond (stage 6 of Figure 5A) for 5 - 7 minutes, and the biochip was prepared after it was washed by PBS.

The key to biochip preparation is self-assembly. If the self-assembling effect is not good, the biological probe cannot be stabilized or rarely fixed on the surface of the biochip. After washing with PBS, the biological probe is easy to dissociate, the SPR response value is obviously decreased, close to the baseline before fixation (stage 5 of Figure 5B), that is, a small number of biological probes were fixed, will reduce the detection sensitivity, and even cause the detection cannot be processed. Therefore, by real-time monitoring of SPR response value in the biochip preparation process can initially determine whether the preparation of biochip is a success. Of course, activation, fixation, and inactivation can all affect the preparation quality of the chip, as the concen-

tration, process time, etc. had been repeated several tests to determine the optimal experimental conditions.

Direct method detection of PACAP

The cell membrane receptor (PAC1R) with a concentration of 300 ppm was immobilized on the surface of the biochip to detect the pituitary adenylate cyclase activating peptide (PACAP-38 modified type, marked PN37R) with concentrations of PN37R was 0.1 mg/L, 0.2 mg/L, 0.3 mg/L, 0.4 mg/L, 0.5 mg/L, 1 mg/L, 2 mg/L, 5 mg/L, 8 mg/L, and 10 mg/L. The binding process of 0.1 - 0.4 mg/L PN37R with PAC1R could not be effectively monitored. That was probably because its concentration was too low. The immune response binding process of 0.4 mg/L PN37R with PAC1R was shown in stage 1 of Figure 6A. The injection of PN37R did not cause an increase in the SPR response value.

The immune response dynamic detection curve of 0.4 mg/L, 0.5 mg/L, 1 mg/L, 2 mg/L, 5 mg/L, 8 mg/L, and 10 mg/L PN37R with the PAC1R were recorded in stages 1, 3, 5, 7, and 9 of Figure 6A, respectively. Each sample was tested for about 200 seconds, and the immune binding was almost saturated after that. Each sample was first washed with PBS buffer and then SDS-HCl was injected to dissociate the antigen-antibody conjugate from the chip surface to achieve chip regeneration. The next sample could be detected when the SPR response value was reduced to the baseline. The stages 2, 4, 6, 8, 10, 12, and 14 in Figure 6A are records of the regeneration process.

When the PN37R samples of 0.5 mg/L, 1 mg/L, 2 mg/L, 5 mg/L, 8 mg/L, and 10 mg/L were pumped into the chip surface in turn, the resonance curves (Figure 6B) obtained during the first SPR scanning were recorded (scanning range 2°). It can be seen that with the increase of the sample concentration, the antigen-antibody conjugate mass on the surface of the biochip increases, the resonance angle increases and the SPR response value increases.

As shown in Figure 6C, the kinetic curves of the PN37R and PAC1R immunoreaction processes were redrawn for ease of analysis and comparison. It can be seen from Figure 6C that the immune response rates of different concentrations of PN37R and PAC1R are fast at first, then slow and gradually saturated. The reaction process is approximately logarithmic and conforms to the immune response rules. And the immune response speed increased as the concentration of PN37R in the samples increased. Each sample was tested for about 200 seconds, and as the immune response progressed, the antigen-antibody conjugate on the surface of biochip increased and the SPR response value increased. If the reaction time of this group of experiments was extended, the curve tended to be gentle, and the immune response saturation trend became more obvious. The detection time of the immunoreaction should be set according to the response value of the antigen-antibody, combined with the actual detection requirements. The longer the immune response time, the more fully the response can theoretically lower the detection limit.

Figure 6D is the standard curve for direct detection of PN37R. At room temperature (20 ± 1°C), the relative response value of when to pass the sample immune response 200 seconds was uesd for the vertical axis, the sample concentration of antibodies was taken as the abscissa. That is to say, the SPR response value of samples in the concentration range of 0.5 - 10 mg/L and the concentration is basically in the linear relationship. The standard curve equation is:

$f(x) = 0.0201\ x - 0.3922$,

the correlation coefficient (R^2) is 0.991. If we have the SPR response value of samples with unknown concentration, then the concentration of PN37R in the sample can be obtained by querying the standard curve. This method is suitable for PACAP antibody screening, antibody affinity, and immune response kinetics research. The detection limit of direct detection of PN37R is up to 0.5 mg/L.

The PN37R standard was diluted with PBS buffer, and the solutions with concentrations of 3 mg/L, 4 mg/L, and 6 mg/L were used as the test samples. The prepared concentrations were recorded as their true values. The sample was passed into the flow cell and immunoreacted with the probe (PAC1R) on the surface plasmon resonance biochip. The surface of the chip was scanned by SPR, and the immune response curve of the tested samples was obtained. The concentration of the sample was calculated according to the standard curve in Figure 6D; then it was used as the detection value. The test value was compared with the real value, and the detailed data were shown in Table 1. The absolute deviation and relative deviation values of the detection value and the true value are low, indicating that the SPR biochip detection system can be used effectively in the quantitative detection of PN37R, and the experimental method is feasible.

Kinetic analysis

Direct detection of PACAP can be used to further calculate the dynamic characteristic parameters to provide information to reveal the dynamics rule between antigen and antibody. In order to fully verify the reliability of the SPR system and the dynamic parameters calculation method established in this paper, the immune response process of PAC1R and PACAP-38 and PK38W were detected by the same method and condition as PN37R direct detection, and the kinetic curves were recorded. The results are shown in Figure 7. The immune responses' kinetic parameters of PAC1R with PN37R, PACAP-38, and PK38W are calculated.

According to formulas (1) and (2), the calculation process of k_a and k_d is:

(1) First, calculate the derivative of response signal R (resonance angle) with respect to the response time t, then a straight line of dR/dt to R can be obtained, the slope is $-(k_a C_A + k_d)$. Suppose: $K_s = -(k_a C_A + k_d)$.

(2) K_s to C_A was used to do the same drawing, and also

obtained a straight line, the slope is k_a and the intercept is k_d.

According to the above mathematical model, the corresponding response value R (take the first paragraph of the data segment) of the largest part of the slope on the dynamic curve was obtained first, then the derivative of R with respect to t was calculated. dR/dt to R was used for linear fitting, and the slope of the fitting curve was calculated. Then the slope to the corresponding antibody (antigen) concentration was used for linear fitting, the slope and the intercept are k_a and k_d. The kinetic parameters' calculation results of immune response of the PAC1R with PN37R, PACAP-38 and PK38W were shown in Table 2.

As shown in Table 2, the magnitude orders of kinetic parameters of the immune response between PAC1R and PACAP-38, PK38W, PN37R are basically the same. However, the specific values of the binding rate constant and dissociation rate constant are slightly different as the molecular weights of PACAP-38, PK38W, PN37R are different. The molecular weight of PK38W and PN37R is about 300 Da more than that of PACAP-38, so the binding rate constant and dissociation rate constant of PK38W and PN37R are slightly larger than that of PACAP-38.

CONCLUSION

The self-developed angle scanning portable SPR sensor was used for PACAP detection and kinetics research. The quantitative detection experiment of PACAP concentration was carried out, the detection limit of direct detection of modified PACAP-38 (PN37R) is up to 0.5 mg/L. The kinetic reactions of PACAP-38, PK38W, PN37R, and PAC1R were studied. The binding rate constant k_a and dissociation rate constant k_d of the ligand and receptor immune response were calculated. Compared with the traditional biochemical analysis method, detection of PACAP with the SPR biochip has a series of advantages, such as label-free, simple sample processing, could quickly give quantitative results, could do real-time detection and study of reaction kinetics, etc. The established device and method are suitable for the detection of PACAP in a large number of samples, the basic research of PACAP, and its PAC1R affinity.

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Declaration of Interest:
All authors have no conflict of interest to declare.

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