ELSEVIER

Biochimica et Biophysica Acta 1512 (2001) 308–316

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

The interaction of trichosanthin with supported phospholipid membranes studied by surface plasmon resonance

Ying-Jie Lu, Xiao-Feng Xia, Sen-fang Sui *

Department of Biological Sciences and Biotechnology, State-Key Laboratory of Biomembrane, Tsinghua University, Beijing 100084, PR China

Received 1 December 2000; received in revised form 13 March 2001; accepted 5 April 2001

Abstract

Trichosanthin (TCS) is a toxic protein isolated from a Chinese herbal medicine, the root tuber of *Trichosanthes kirilowii Maximowicz* of the Curcurbitaceae family. It is now used in China to terminate early and mid-trimester pregnancies. The ribosome inactivating property is thought to be account for its toxicity; it can inactivate the eukaryotic ribosome through its RNA N-glycosidase activity. The interactions of TCS with biological membrane is thought to be essential for its physiological effect, for it must get across the membrane before it can enter the cytoplasm and exert its RIP function. In the present work, the interaction of TCS with supported phospholipid monolayers is studied by surface plasmon resonance. The results show that electrostatic forces dominate the interaction between TCS and negatively charged phospholipid containing membranes under acid condition and that both the pH value and the ionic strength can influence its binding. It is proposed that, besides electrostatic forces, hydrophobic interaction may also be involved in the binding process.

Keywords: Trichosanthin; Surface plasmon resonance; Supported monolayer; Lipid–protein interaction

1. Introduction

Trichosanthin (TCS), a type-I ribosome-inactivating protein (RIP) isolated from the root tuber of *Trichosanthes kirilowii Maximowicz*, has long been used in China to terminate early and mid-trimester pregnancies and to treat trophoblastic tumors because it can bind to the trophoblastic syncytial layer and selectively kill syncytiotrophoblast cells [1]. TCS consists of a single chain peptide with no carbohydrate and phosphate group, which shows sequence homology with subunit A of ricin-D [2]. The three-dimensional structure of TCS has been determined to a resolution of 1.88 and 1.73 Å, respectively, by two groups [3,4]. TCS has been found to possess a variety of biological activities including anti-cancer [5,6] and anti-HIV-infection [7–9]. In the early 1990s, TCS was applied in the treatment of patients with AIDS or AIDS-related complex in phase I and II studies [10–13].

Surface plasmon resonance (SPR) has recently emerged as a useful tool in obtaining thermodynam-

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DMPS, dimyristoylphosphatidylserine; DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; NaAc-HAc, sodium acetate–acetic acid; SPR, surface plasmon resonance; TCS, trichosanthin

* Corresponding author. Fax: +86-10-6278-4768;
E-mail: suisf@mail.tsinghua.edu.cn

0005-2736/01/$ – see front matter
PII: S0005-2736(01)00334-0

ics and kinetics information on biomolecular interactions for its label-free and real-time measurement. It is particularly useful for processes occurring at or near interface [14]. SPR biosensors can translate a biospecific interaction between a ligand in solution and a binding site on the sensor surface into a detectable signal, and the supported planar lipid film is a widely used one among the sensor family [14,15]. It allows the evaluation of the structural properties of such films and permits the measurement of the mass of deposited material with a high degree of accuracy. The first application of this method involved an analysis of arachidate monolayer assemblies on silver film [16]. Since that time, SPR sensor based on solid supported planar lipid film has been extensively used to characterize lipid layers [17,18], protein–protein interactions [19,20], DNA–protein interactions [21,22], and also membrane–protein interactions [23–26]. Both the binding constant and the stoichiometry of the interaction can be obtained by SPR.

TCS belongs to the ribosome inactivating protein (RIP) family, which can inactivate ribosome by removing A_{4324} of 28S rRNA via *N*-glycosidase activity [27,28]. Though it was proposed that TCS can enter cells via a LRP or megalin mediated endocytosis [29], it is still a puzzle how TCS enters cytoplasm and exerts its toxicity since TCS is packaged in endosomes by endocytosis. As RIP must enter cytoplasm before it can show ribosome-inactivating activity, TCS also needs to escape from the vesicle and enters the cytoplasm after endocytosis. Therefore, It is important to study the TCS/phospholipid interaction in order to investigate the mechanism involved. Our previous work [30,31] has shown that TCS can insert into lipid monolayer in a pH-dependent manner, and the insertion is also phospholipid dependent. TCS selectively inserts into negatively charged phospholipids while not for neutral ones. To further investigate these phenomena, in the present work we combined SPR and supported planar membranes to study the binding process and to characterize the factors affecting the binding between TCS and membranes. Our results show that TCS prefers to bind to negatively charged phospholipid-containing membranes and such binding is dependent on the pH value and the ionic strength. Our results also indicate that both electrostatic and hydrophobic forces may be involved in the TCS binding.

2. Materials and methods

2.1. Materials

Trichosanthin was purified from the root tuber of *Trichosanthes kirilowii* [32], and the purity was examined as a single band by sodium dodecyl sulfate–polyacrylamide gel electrophoresis in terms of silver stain. DPPC, DPPG, DMPS and DMPC were purchased from Sigma Chemical Co. and used without further purification. Other chemicals were purchased locally.

2.2. Preparation of supported monolayers

Supported membranes constitute a widely used model membrane system in studying lipid–protein interactions [33]. In the present work, supported monolayers on gold-coated cover slides were prepared according to the methods described by Sui et al. [26]. Briefly, the cover slides were cleaned in a mixture of 20 ml water, 20g $K_2Cr_2O_7$ and 180 ml H_2SO_4 (98%) for 24 h, and extensively rinsed with water (distilled and deionized), then dried in air. The cleaned slides were hydrophobilized by soaking in a mixture of dimethyldichlorosilane and chloroform (1:9) for 30 min and rinsing with chloroform and ethanol. Then, a 50-nm thick gold film was deposited onto the slides under vacuum condition.

A computer-controlled LB trough was used to prepare monolayers. The LB trough had a volume of 400 ml and a surface area of 370 cm^2. A movable barrier was used to change the area that was covered by the lipid monolayer, and a Willhelmy system was used to measure the surface tension. Phospholipids dissolved in an organic solution (chloroform/methanol, v/v = 3:1) were carefully spread on the surface of water in the trough. After 30 min, the monolayer was compressed by the moveable barrier to a surface pressure of 40 mN/m, and then horizontally transferred onto a gold-covered slide by hand. All experiments were performed at room temperature (25 ± 1°C).

2.3. Instrument setup

A home-made SPR system [26,34] based on the Kretschman configuration was used in the present

work (Fig. 1). A semiconductor HL6711G laser (wavelength of 670 nm) was used as the incident polarized light source, and a photodiode served as a detector to collect the reflected light. The chip was stuck to a prism with a refractive index of 1.8 through an index matching oil (cedar wood oil, $n = 1.515$). A 200 μl sample chamber was made out of Teflon, which was rotated by a computer-controlled step motor with a minimum step angle of 1/36 000°. The numerical evaluation of each resonance angle has an error of less than 0.001°. Each curve of response unit (the angle change of φ, unit 0.001°) vs. time was measured by varying the incident angle around the resonance point.

2.4. Theory

The interaction between lipid and protein is a complex process, and several theoretical models have been developed to treat this problem [35–37]. For analyzing the binding data of TCS to supported monolayers, the time course of TCS binding to membrane was fitted with a pseudo-first-order kinetics model [38–40], as shown in Fig. 2. The simulated curve fitted the binding curve well, with a coefficient of curve fitness of $R^2 = 0.99$. So the TCS binding process can be analyzed as one-step association–dissociation equilibrium:

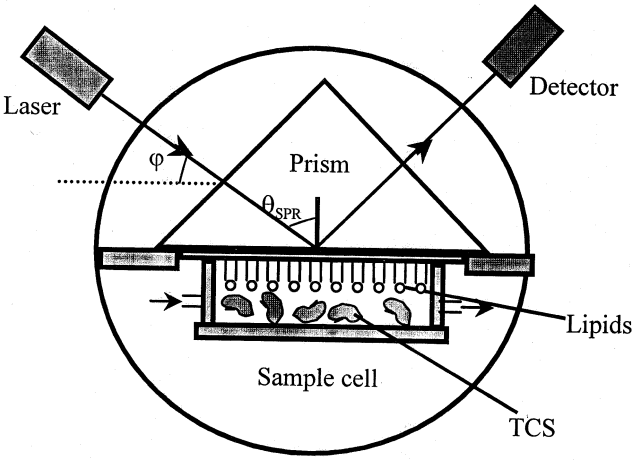


Fig. 1. Schematic illustration of the arrangement of the surface plasmon resonance setup. The triangle prism (refractive index 1.8) is fitted onto a small rotating angle measuring apparatus. In this system the angle φ is directly measured. Values of θ_{SPR} are derived [34] from the formula $\theta_{SPR} = 45° + \arcsin[1/n_0 \cdot \sin(45° - \varphi)]$, where n_0 is the refractive index of the prism. The angular accuracy of the measuring apparatus is 0.001°. The volume of the sample cell is 200 μl.

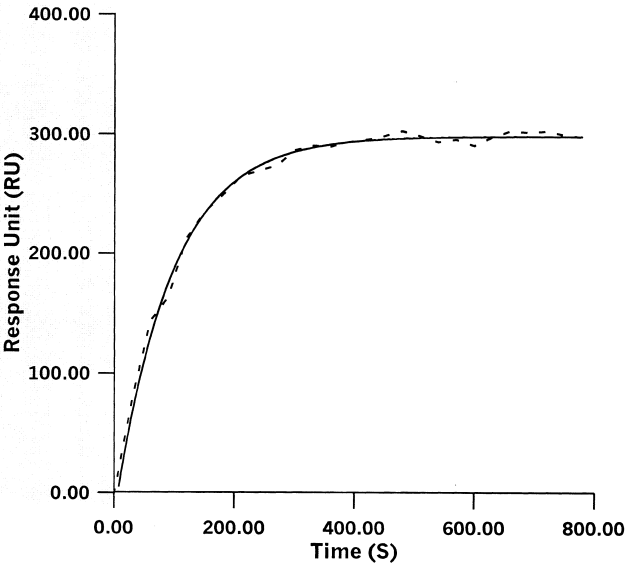


Fig. 2. The real-time SPR curve of TCS binding onto DPPG monolayer and the simulated curve. The binding curve (broken line) is carried out in a buffer of 0.05 M Na^+, pH 4.0. The bulk concentration of TCS is 2.22×10^{-6} M. The simulated curve (solid line) is fitted according to a pseudo-first-order kinetics model. Coefficient of curve fitness: $R^2 = 0.99$.

$$\begin{array}{ccccc} A_f + & L & \underset{k_d}{\overset{k_a}{\leftrightarrow}} & A_b \\ A_0 & B_0 & & C_0 \\ A & B & & C \end{array}$$

where L represents a cluster of lipid molecules in the lipid monolayer covered by one TCS molecule, which can be regarded as a binding site; A_f, free TCS in the buffer; A_b, TCS associated with lipid molecules; k_a, the association rate constant; k_d, the dissociation rate constant. A_0, B_0, C_0 are the initial concentrations; A, B, C represent the concentrations of A_f, L, A_b, respectively, at the time when the system has reached equilibrium (all concentrations are expressed as standard molar concentration). Consequently, an equation concerning the binding constant K_A can be expressed as:

$$K_A = \frac{k_a}{k_d} = \frac{C}{AB} = \frac{C}{A(B_0 - C)} \quad (1)$$

Rearrangement of the equation shows that:

$$\frac{1}{C} = \frac{1}{B_0 K_A A} + \frac{1}{B_0} \quad (2)$$

Since B_0 and K_A are both constants, there is a linear relationship between $1/C$ and $1/A$. The numerical value of C can be calculated from the SPR angle (θ_{SPR}) change. According to Stenberg et al. [41], we can calculate the surface concentration of TCS using the following equation:

$$\sigma = 10\Delta\theta_{SPR} \quad (3)$$

where σ is the surface concentration (ng/mm^2), $\Delta\theta_{SPR}$ is the SPR angle change (degrees). Then, we can obtain TCS surface molar concentration C (mol/m^2) by

$$C = \sigma \times 10^{-3}/M \quad (4)$$

M is the molar mass of TCS.

The numerical value of A can be obtained by the relationship $A = A_0 - C \cdot S/V$, where S is the surface area (m^2) covered by buffer and V is the volume (liters) of buffer solution.

Thus, we can obtain the dissociation constant K_D from the following equation:

$$K_D = 1/K_A = \text{Slope}/\text{Intercept} \quad (5)$$

where both the values of Slope and Intercept can be measured by Eq. 2.

The experimental procedure to measure K_D can be described briefly as the following: At first, 200 μl buffer was added into the chamber. After a 20-min equilibration period, a certain amount of TCS was added into the chamber and mixed homogeneously. The SPR curve was recorded once each minute. Every 20 min another aliquot of protein was added to increase the total protein concentration. The data were collected and then analyzed according to the equations above.

3. Results

3.1. Low pH can induce TCS to bind to the anionic monolayers

It is supposed that the interaction of TCS with phospholipid membranes may refer to a complex process, in which the pH effect may be involved [30,31]. As shown in Fig. 3, the maximum surface concentration of TCS on DPPG monolayer by adding excessive TCS is strongly dependent on the pH of the buffer. At pH 7 the surface concentration of the membrane-bound TCS is around 2.06 ± 0.148nmol/m^2. With decreasing pH, the surface concentration of the bound TCS increased and reached its maximum value (about 50.9 ± 0.741nmol/m^2) around the pH value of 4. Comparing with the surface concentration at pH 7, this value is about 25-fold increased. As the pH value of the buffer decreased further; however, the surface concentration of membrane-bound TCS became small, reaching a value of 24.4 ± 4.07nmol/m^2 at pH 3, about half of the amount bound at pH 4. In the case of TCS binding to a DMPS monolayer, the pH effect on surface concentration of membrane-bound TCS follows a similar manner as that observed on the DPPG monolayer (results not shown). When TCS binds to a DPPC monolayer, however, the surface concentration of membrane-bound TCS is not sensitive to a change in pH value in the region from 7 to 3, and it

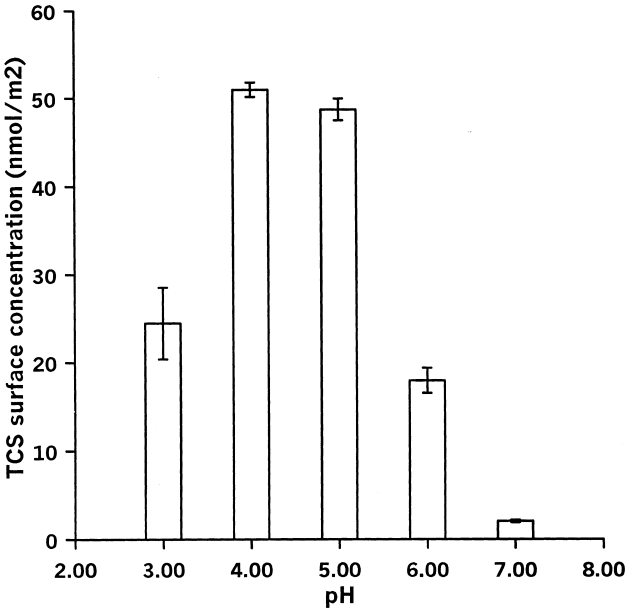
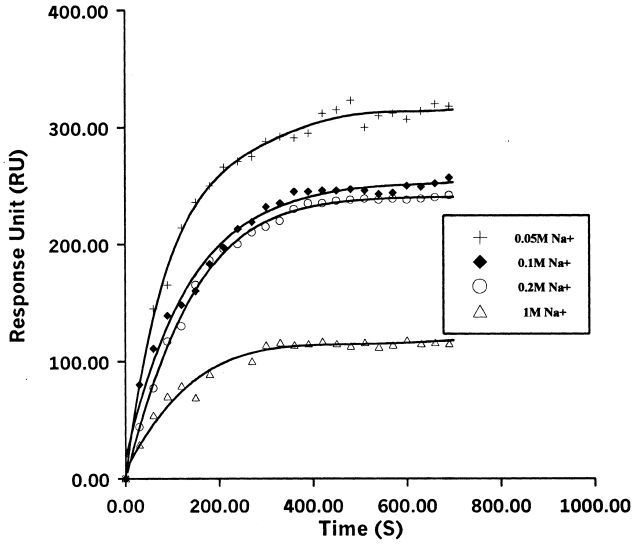
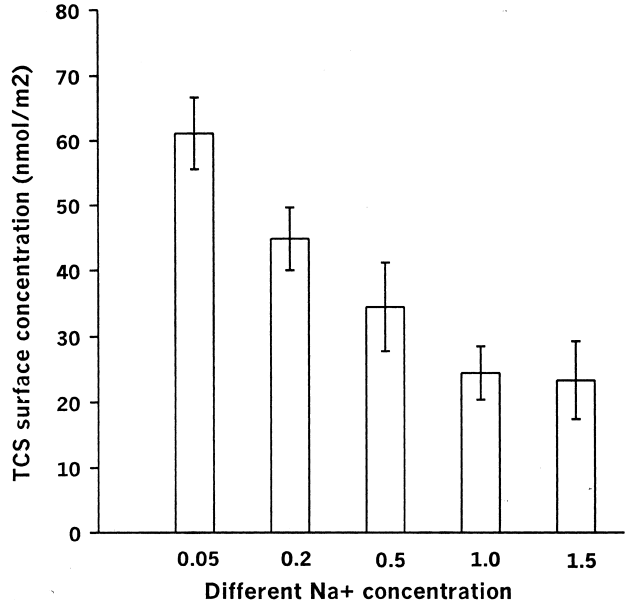


Fig. 3. The surface concentrations of TCS on DPPG monolayer under different pH values. The buffer is 0.2 M NaAc-HAc with pH varying from 3 to 7. The bulk concentration of TCS is 2.22×10^{-6}M. All experiments were performed at room temperature (25 ± 1°C).



(a)



(b)

Fig. 4. (a) The time-dependent SPR response curves of TCS on DPPG monolayers under different concentration of Na^+. RU is the unit used to express the SPR signal; one RU equals a 0.001° angular change in φ. (b) The relationship between the surface concentration of membrane-bound TCS and the Na^+ concentration in the eluting buffer. The bars represent the TCS surface concentration after detachment. The bulk concentration of TCS is 2.22×10^{-6}M. All experiments were performed at room temperature (25 ± 1°C).

has a nearly constant value of about 1.48 ± 0.148 nmol/m^2, which is not obvious.

3.2. The binding of TCS to the monolayer is influenced by ionic strength

The results mentioned above indicate that the electrostatic interaction is an important factor in the binding process of TCS to phospholipid monolayers. Since the ionic strength of the environment can influence the electrostatic interaction, it can be assumed that changing ionic strength may change the binding property of TCS. So we examined the effect of ionic strength on TCS binding in the following experiments. Since a maximum surface concentration of TCS was obtained at pH 4, buffers with pH 4 were used in the measurements. Fig. 4a shows the dynamic process of TCS binding to the monolayers under different Na^+ concentration. It can clearly be seen that with increasing Na^+ concentration, both the binding rate of TCS to the membrane and the maximum surface concentration of membrane-bound TCS decrease. According to Bondeson et al. [42], we calculated the numerical values of the binding rate constants. The constants and the maximum surface concentrations of TCS under different Na^+ concentrations are shown in Table 1. The detachment effect of membrane-bound TCS by varying Na^+ concentration was also measured. After the surface concentration of membrane-bound TCS reached its maximum value in 0.05 M Na^+ buffer, the membrane-bound protein was eluted by using buffer with higher ionic concentration increased step by step. As shown in Fig. 4b, with increasing ionic

Table 1
The association rate constant k_a and maximum surface concentration of TCS on DPPG monolayers in different Na^+ concentration buffers

Na^+ concentration (M)	k_a ($M^{-1}\,s^{-1}$)	Maximum TCS surface concentration (nmol/m^2)
0.05	3.68×10^3	61.1 ± 5.56
0.1	2.44×10^3	53.0 ± 4.73
0.2	2.39×10^3	50.9 ± 0.741
1	0.92×10^3	24.4 ± 4.07

The buffer's pH is 4.0 and the bulk concentration of TCS is 2.22×10^{-6} M. All experiments were performed at room temperature (25 ± 1°C).

strength more membrane-bound TCS was detached at beginning and then it reached to equilibrium as the concentration of Na^+ increased to 1 M. With increasing Na^+ concentration further, about half of surface bound TCS still remains on the membrane.

3.3. Binding of TCS depends on the charges of the monolayers

The main difference between DPPC, DPPG and DMPS lies in the charge of the head-group: while the head-group of DPPC is neutral, those of DPPG and DMPS are negatively charged. It can be imagined that the binding of TCS to phospholipid monolayers is depending on the surface charges of the latter. In order to examine this supposition, we investigated the effect of membrane surface charge density on the affinity of TCS for the membranes. Fig. 5 exhibits the relationship between the surface concentration of membrane-bound TCS and the molar fraction of DPPG in DPPG/DPPC mixtures (Fig. 5a) and the relationship in DMPS/DMPC mixtures (Fig. 5b). In both cases, the surface concentration of membrane-bound TCS clearly shows a linear correlation to the mole fraction of DPPG and DMPS, respectively. The surface concentration of membrane-bound TCS increases with the increasing molar fraction of negatively charged phospholipid. A maximum surface concentration 50.9 nmol/m^2 of TCS is observed as it binds to the pure DPPG monolayer, so we can calculate the average surface area occupied by a TCS molecule to be about 3260 $Å^2$. Since each DPPG molecule occupies an area of 48 $Å^2$ [43] at a surface pressure of 40 mN/m, the molecular ratio between membrane-bound TCS and DPPG at the monolayer surface is about 1:68. While for another anionic phospholipid DMPS, the maximum surface concentration of TCS is 57.0 nmol/m^2 (see Fig. 5b), a little higher than that on pure DPPG. Since DMPS molecule occupies an area of 40 $Å^2$, the molecular ratio of TCS and DMPS is 1:73, which is close to the ratio on DPPG containing monolayer. So it can be concluded that the binding amount of TCS to the monolayer is unspecific to the species of anionic phospholipid, but critically depends on the surface density of negatively charged phospholipid.

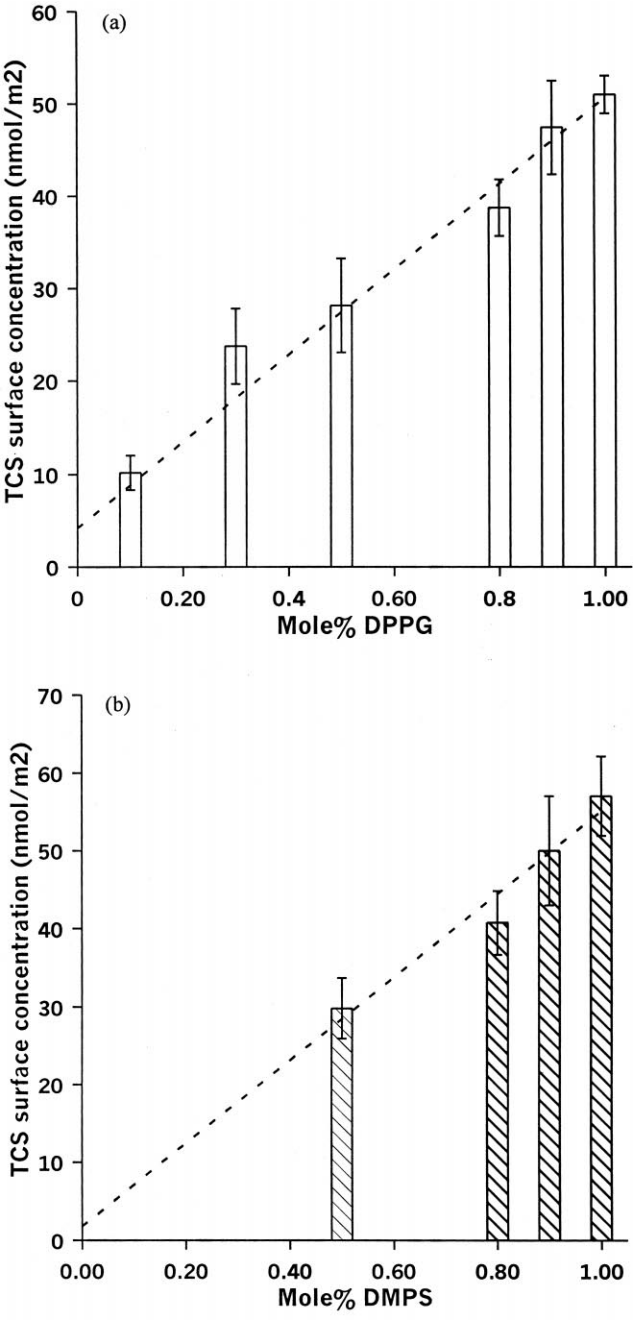


Fig. 5. The surface concentration of TCS on mixed monolayer with varying percentage of negatively charged phospholipids. The bars represent the surface concentration of TCS, and the dashed lines represent the tendency of the change in surface concentration of TCS. The buffer is 0.2 M NaAc-HAc (pH 4.0), and the bulk concentration of TCS is 2.22×10^{-6} M. (a) DPPG/DPPC monolayer mixtures with DPPG's molar percentage 10%, 30%, 50%, 80%, 90%, and 100%, respectively. (b) DMPS/DMPC monolayer mixtures with DMPS's molar percentage 50%, 80%, 90%, and 100%, respectively. All experiments were performed at room temperature (25 ± 1°C).

The dissociation constants between TCS and monolayers with different content of negatively charged phospholipid were also determined by SPR. Data obtained from measurements were analyzed according to Eq. 5, and the numerical values of the dissociation constants are shown in Table 2. Since the membrane-bound TCS may exist in different states, for example, salt-dependent and salt-independent, what we obtain from Eq. 5 is an apparent dissociation constant which contains a composite effect of the different states.

4. Discussion

The pH value plays an important role in the interaction of toxins with cell membranes. Low pH can induce membrane insertion for many toxins [44]. In the present work, we found that the binding of TCS to anionic phospholipid membranes also preferentially occurs in an acidic environment. As shown in Fig. 3, when the pH value of the buffer is 7, the binding of TCS is negligible to the phospholipid monolayer. While as the pH value is lower than 7, the surface concentration of TCS will increase significantly. This phenomenon is interpretable according to the electrostatic properties of both lipid membrane and TCS. On the side of lipid monolayer, an average value of pK_a as 2.5 for DPPG was taken [45]. If the phospholipid is considered as a mono-acid, we can obtain that more than 96% of DPPG molecules carry one negative charge in the pH range from 7 to 4. This result indicates that the surface charge density of DPPG monolayer does not change much in this pH range. On the other side, TCS itself will carry more positive charges as the pH value varies from 7 to 4 since TCS has a pI of 9.4. As a result more TCS molecules bind to the monolayer when pH decreased. When the pH is decreased to 3, however, the amount of the negatively charged DPPG will decrease to 75% of the total phospholipid. This change in the amount of negative charge at the monolayer surface may cause the binding amount of TCS to drop down.

Table 2
The dissociation constants (K_D) measured on different ratio of negatively charged phospholipids

Mol% of DPPG	K_D (M)
30	$7.85 \pm 0.437 \times 10^{-8}$
50	$9.63 \pm 1.41 \times 10^{-8}$
80	$8.90 \pm 2.07 \times 10^{-8}$
90	$11.6 \pm 1.19 \times 10^{-8}$

Running buffer is 0.2 M NaAc-HAc, pH 4.0. All experiments were carried out at room temperature (25 ± 1°C).

Further direct evidence to interpret the role of the electrostatic force in the TCS binding process comes from the experimental results shown in Fig. 5. The surface concentration of membrane-bound TCS has a nearly linear correlation with the molar fraction of negatively charged lipid in the mixtures. This finding indicates that the electrostatic force indeed dominates the interaction between TCS and phospholipid monolayer.

Although electrostatic force plays essential role in the TCS binding process, hydrophobic interaction may also be involved in the binding process. The results of the detachment effect of high ionic strength buffer on the TCS binding have an inspiration to us. On one side, the results from Fig. 4b indicate that the electrostatic interactions occur in the TCS binding process due to the clear detachment effect of ion. On the other side, from the same measurements we can see that increasing the ionic concentration cannot detach all the membrane-bound TCS. About half of the membrane-bound TCS still remains on the membrane even the Na^+ concentration reaching to 1.5 M. This result indicates that besides electrostatic interaction, another factor, most probably hydrophobic force, takes part in the binding process of the protein. There is another evidence to support this opinion. As shown in Table 2 the data of the dissociation constants of TCS are not sensitive to the molar fraction of negatively charged phospholipid in the mixtures. This phenomenon cannot be explained in terms of electrostatic interactions. In our previous work we have reported that low pH will induce TCS to undergo a tertiary conformational change and more hydrophobic sites will be exposed [31]. Therefore, it makes it possible for TCS to interact with the negatively charged phospholipid-containing monolayers under acid conditions by hydrophobic interactions.

It has been reported [46] that membrane–protein interaction plays important role in RIP's physiolog-

ical effect, so the interaction between TCS and membrane can be important also for TCS to play its role. Although there is only less than 20% negatively charged phospholipids in biological membranes, a phase separation in liquid crystalline mixtures of zwitterionic lipid and acidic lipid can also be induced by changes in pH. The acidic phospholipid-rich domains can presumably be formed [47]. So an enrichment of TCS can occur at the interface of membrane at low pH, and this may help TCS to have a further interaction with membranes.

Acknowledgements

This work was supported by the National Nature Sciences Foundation of China.

References

[1] L. Law, P.P.L. Tam, H.W. Yeung, J. Reprod. Fertil. 69 (1983) 597–604.
[2] X.J. Zhang, J.H. Wang, Nature 321 (1986) 477–478.
[3] K.J. Zhou, Z.J. Fu, M.H. Chen, Y.J. Lin, K.H. Pan, Proteins Struct. Funct. Genet. 19 (1994) 4–13.
[4] B. Gao, X.Q. Ma, Y.P. Wang, S.Z. Chen, S. Wu, Y.C. Dong, Sci. China Ser. B 37 (1994) 59–73.
[5] Y. Huang, Chin. J. Integ. Tradition. West. Med. 7 (1987) 154–155.
[6] S.W. Tsao, K.T. Kan, H.W. Yeung, Toxicon 24 (1986) 831–840.
[7] T.K. Au, R.A. Collins, T.L. Lam, T.B. Ng, W.P. Fong, D.C. Wang, FEBS Lett. 471 (2000) 169–172.
[8] M.S. McGrath, K.M. Hwang, S.E. Caldwell, I. Gaston, K.C. Luk, P. Wu, V.L. Ng, S. Crowe, J. Danniels, J. Marsh, J. Deinhart, P.V. Lekas, J.C. Vennari, H.W. Yeung, J.D. Lifson, Proc. Natl. Acad. Sci. USA 86 (1989) 2844–2848.
[9] J.O. Kahn, K.J. Gorelick, G. Gatti, C.J. Arri, J.D. Lifson, J.G. Gambertoglio, A. Bostrom, R.L. Williams, Antimicrob. Agents Chemother. 38 (1994) 260–267.
[10] V.S. Byers, A.S. Levin, A. Malvino, L. Waites, R.A. Robins, R.W. Baldwin, AIDS Res. Hum. Retroviruses 10 (1994) 413–420.
[11] R.A. Mayer, P.A. Sergios, K. Coonan, L. O'Brien, Eur. J. Clin. Invest. 22 (1992) 113–122.
[12] V.S. Byers, A.S. Levin, L.A. Waites, B.A. Starrett, R.A. Mayer, J.A. Clegg, M.R. Price, R.A. Robins, M. Delaney, R.W. Baldwin, AIDS 4 (1990) 1189–1196.
[13] J.O. Kahn, L.D. Kaplan, J.G. Gambertoglio, D. Bredesen, C.J. Arri, L. Turin, T. Kibort, R.L. Williams, J.D. Lifson, P.A. Volberding, AIDS 4 (1990) 1197–1204.
[14] S.F. Sui, C.D. Xiao, Y. Zhou, W.Z. Xie, J.F. Liang, Adv. Biosensors 4 (1999) 123–137.
[15] Z. Liu, H. Qin, C.D. Xiao, C.H. Wen, S.P. Wang, S.F. Sui, Eur. Biophys. J. 24 (1995) 31–38.
[16] I. Pockrand, J.D. Swalen, J.G. Gordon, M.R. Philpott, Surf. Sci. 74 (1977) 237–244.
[17] L. Haussling, H. Ringsdorf, F.J. Schmitt, W. Knoll, Langmuir 7 (1991) 1837–1840.
[18] Ch. Striebel, A. Brecht, G. Gauglitz, Biosensors Bioelectron. 9 (1993) 139–146.
[19] H. Morgan, D.M. Taylor, C. D'Silva, Thin Solid Films 209 (1992) 112–126.
[20] A. Toth, E. Kiss, F.W. Herberg, P. Gergely, D.J. Hartshorne, F. Erdodi, Eur. J. Biochem. 267 (2000) 1687–1697.
[21] D.R. Mernagh, P. Janscak, K. Firman, G.G. Kneale, Biol. Chem. 379 (1998) 497–503.
[22] P.Y. Tsoi, J. Yang, Y.T. Sun, S.F. Sui, M.S. Yang, Langmuir 16 (2000) 6590–6596.
[23] J.L. Solulages, Z. Salamon, M.A. Wells, G. Tollin, Proc. Natl. Acad. Sci. USA 92 (1995) 5650–5654.
[24] Z. Salamon, G. Tollin, Biophys. J. 71 (1996) 858–867.
[25] S. Hoyse, O.P. Ernst, Z. Dienes, K.P. Hofmann, H. Vogel, Biochemistry 37 (1998) 507–522.
[26] S.F. Sui, Y.T. Sun, L.Z. Mi, Biophys. J. 76 (1999) 333–341.
[27] J.S. Zhang, W.Y. Liu, Nucleic Acids Res. 20 (1992) 1271–1275.
[28] S. Wu, X.H. Lu, Y.R. Zhu, J. Yang, Y.C. Dong, Sci. China Ser. C Life Sci. 41 (1998) 174–180.
[29] W.L. Chan, P.C. Shaw, S.C. Tam, C. Jacobsen, J. Gliemann, M.S. Nielsen, Biochem. Biophys. Res. Commun. 270 (2000) 453–457.
[30] X.F. Xia, S.X. Wang, J.B. Luo, N.S.W. Richy, S.F. Sui, Chin. Sci. Bull. 44 (1999) 1892–1895.
[31] X.F. Xia, S.F. Sui, Biochem. J. 349 (2000) 835–841.
[32] Y. Zhang, W.L. Li, Q. Hao, G. Yu, Q.S. Li, Q.Z. Yao, Biochem. Biophys. Res. Commun. 197 (1993) 407–414.
[33] E. Sackmann, Science 271 (1996) 43–48.
[34] X. Caide, S.F. Sui, Eur. Biophys. J. 28 (1999) 151–157.
[35] T.E. Thorgeirsson, Y.G. Yu, Y.K. Shin, Biochemistry 34 (1995) 5518–5522.
[36] R.M. Peitzsch, M. Eisenberg, K.A. Sharp, S. Mclaughlin, Biophys. J. 68 (1995) 729–738.
[37] D. Murray, L. Hermida-Matsumoto, C.A. Buser, J. Tsang, C.T. Sigal, N. Ben-Tal, B. Honig, M.D. Resh, S. Mclaughlin, Biochemistry 37 (1998) 2145–2159.
[38] E.E. Loomans, T.A. Beumer, K.C. Damen, M.A. Bakker, W.J. Schielen, J. Colloid Interface Sci. 192 (1997) 238–249.
[39] I.V. Polozov, A.I. Polozova, V.K. Mishra, G.M. Anantharamaiah, J.P. Segrest, R.M. Epand, Biochim. Biophys. Acta 1368 (1998) 343–354.
[40] M.A. Cooper, J. Carroll, E.R. Travis, D.H. Williams, D.J. Ellar, Biochem. J. 333 (1998) 677–683.

[41] E. Stenberg, B. Persson, H. Roos, C. Urbaniczkoy, J. Colloid Interface Sci. 143 (1991) 513–526.
[42] K. Bondeson, A. Frostell-Karlsson, L. Fagerstam, G. Magnusson, Anal. Biochem. 214 (1993) 245–251.
[43] D. Chapman, D.F.H. Wallach, Biological Membranes, Academic Press, London, 1973, pp. 8–9.
[44] C. Lesieur, B. Vécsey-Semjén, L. Abraml, M. Fivaz, F. Gisou van der Goot, Mol. Membr. Biol. 14 (1997) 45–64.
[45] J.F. Tocanne, J. Teissie, Biochim. Biophys. Acta 1031 (1990) 111–142.
[46] B. Beaumelle, M. Alam, C.R. Hopkins, J. Biol. Chem. 268 (1993) 23661–23669.
[47] P. Yeagle, The Structure of Biological Membranes, CRC Press, London, 1991, pp. 122–123.