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Effect of phospholipid on trichosanthin adsorption at the air–water interface

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Abstract

Trichosanthin (TCS) is a toxic protein with multiple pharmacological properties. It belongs to the type I ribosome inactivating protein (RIP) family and can inactivate the eukaryotic ribosome through its RNA *N*-glycosidase activity. The interaction between TCS and phospholipid membrane was thought to be essential for its physiological effect, for it must get across the cell membrane before it can enter the cytoplasm and exert its RIP function. In order to study the TCS–phospholipid interaction, the difference between spontaneous and phospholipid induced adsorption of TCS at the air–water interface was investigated, and the results were analyzed according to the diffusion–penetration–rearrangement adsorption model. The results showed that both negatively charged 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG) and neutral 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine can accelerate the adsorption rate, while there exists a possible membrane induced conformational change of TCS which is specific for the negatively charged DPPG. We also proposed a revised model for the diffusion controlled initial adsorption period.

Keywords: Trichosanthin; Protein adsorption; Phospholipid–protein interaction; Air–water interface

1. Introduction

Trichosanthin (TCS) is an active component isolated from a Chinese herbal medicine Tianhuafen (the root tuber of *Trichosanthes kirilowii maxim*, Cucurbitaceae) [1,2]. It has long been used clinically in China to terminate early and midtrimester pregnancies [3] and to treat trophoblastic tumors [4]. Recent studies have revealed a broad spectrum of other biological and pharmacological properties of TCS, including anti-HIV [5–7] and DNA topoisomerase activity [8]. In the early 1990s, TCS was applied in the treatment of patients with AIDS or AIDS-related complex in phase I and II studies [9–12].

Trichosanthin belongs to the type I ribosome inactivating protein (RIP) family [13,14], it consists of a single chain (27 kDa, 247 amino acids) [15] which shows sequence homology to the A chain of many type II RIPs [15,16]. The three-dimensional structure of TCS has been resolved to 1.73 and 2.6 Å by two groups [17,18], and both the genomic and cDNA of TCS have been cloned [19,20].

Many studies have revealed the fact that membrane–protein interaction plays an important role in RIP's physiological effect [21,22], because these heterogeneous proteins must be translocated across the biological membrane before they can meet the

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ribosome and then inactivate it. Our previous work has shown that TCS can penetrate into the negatively charged phospholipid monolayer in a pH-dependent manner and a membrane induced conformational intermediate exists in the membrane insertion process [23,24].

The air–water interface provides a simple but effective model for the studies of protein hydrophobicity, the kinetics of hydrophobic interaction and the conformational change in the interaction process [25]. Based on the studies on a series of proteins with different tertiary structures, Graham and Phillips [26–30] summarized the adsorption process to consist of the following three steps: (1) the initial diffusion controlled adsorption, in which protein adsorbs to the interface whenever it collides with the surface; (2) the penetration of proteins into the preexisting protein layer at the interface; in this process the increase in protein surface concentration leads to an increase in surface pressure; (3) the conformational rearrangement of the proteins in the adsorbed layer; in this process the increase in surface pressure is rather caused by a conformational change of the adsorbed proteins, while the protein surface concentration changes little.

In the present work, both the spontaneous and phospholipid induced adsorption of TCS to the air–water interface were studied using a Wilhelmy plate method [31]. The resulting π–t curves were recorded and analyzed using the adsorption model mentioned above. From the differences between the adsorptions in the absence and presence of phospholipid induction, the interactions between TCS and phospholipids were deduced.

2. Materials and methods

2.1. Chemicals

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG) were purchased from Sigma (St. Louis, MO, USA). The other chemicals used were of analytical grade made in China. The deionized pure water used in the experiment had a resistivity of no less than 18.0 MΩ·cm. For experiments at different pH, 50 mM Tris–HCl buffer was used for pH 7.4, while 50 mM sodium acetate–acetic acid (NaAc–HAc) buffer was used for pH 4.6. All the solutions were freshly prepared.

2.2. Preparation of TCS

TCS was extracted form the root of *T. kirilowii* (Tianhuafen) according to Zhang et al. [32] with slight modification. The dried slice of Tianhuafen obtained from a local drugstore was homogenized with 50 mM Tris–HCl buffer at pH 6.8 (buffer A) using a high speed blender. The outcome was centrifuged to remove the insoluble material. Ammonium sulfate was added to 40% saturation to the supernatant, the mixture was left for 12 h and centrifuged. The collected supernatant was adjusted to 75% saturation with ammonium sulfate, left for 6 h, and centrifuged. The precipitate was resuspended with buffer A, and dialyzed overnight against buffer A. The resulting solution was applied to a CM-Sepharose C-50 column, washed with buffer A, and eluted with buffer A containing 0.3 M NaCl. The elution peak was collected, and put onto the second column of Sephadex G-75 which was equilibrated with buffer A, and eluted under the same conditions. TCS appeared in the second elution peak. Purity determination of TCS showed a single band at the 27 kDa position on SDS–PAGE (silver stain).

2.3. Surface pressure measurement

The surface pressure (π) increase caused by protein adsorption was measured using the Wilhelmy plate method with a NIMA 9000 (Nima Technology, Science Park, Coventry, UK) microbalance. Surface pressure is defined as the surface tension difference before and after protein layer was formed on the solution surface. All the data were automatically collected and recorded by a personal computer. The home-made Teflon sample trough [33] had a volume of 4 ml and a surface area of 10 cm^2. The subphase bulk was well stirred with a magnetic bar. Before the experiment, the sample trough was thoroughly cleaned until a constant surface tension value of 72 mN/m was reached for pure water. During the experiment, TCS was injected into the subphase through a side sample hole. The pressure change was then followed for the indicated time. In phos-

pholipid induced adsorption experiments, 0.5 nmol indicated phospholipid (DPPC or DPPG) was spread on the buffer surface and left for 1 h before TCS was injected. So the area per molecule of the phospholipids was about 300 Å^2. The temperature of the system was maintained at 25 ± 0.5°C.

2.4. Measurement of π–Γ isotherm

A KSV5000 LB trough (KSV instruments, Finland) was used to obtain the surface pressure–surface concentration (π–Γ) isotherms of protein layers at the air–water interface. The Teflon trough being 270 mm long and 75 mm wide was placed on an isolated vibration-free table and enclosed in a glass chamber to avoid contaminants from the environment. Temperature regulation of the trough was controlled by circulation of constant temperature water from an external circulator through the tubes attached to the aluminum based plate of the trough. The trough was thoroughly cleaned before the experiment until the measured surface pressure was below 0.1 mN/m upon complete compression for pure water. During the experiment, 180 ml of 2 nM TCS solution was added to the trough and left for 10 h for equilibrium before compression. The π–Γ curve was obtained by compressing the protein layer at the air–water interface with two Teflon barriers at a speed of 1.5 cm^2/min at 25 ± 0.5°C, the total area of the trough is 202.5 cm^2.

Each experiment was carefully repeated with good reproducibility, deviation within ± 5%.

3. Results

As pointed out by Erickson et al. [34], because the denaturation of protein continuously happens at the interface, no true equilibrium state can be established in the protein adsorption process. In our experiment, the surface pressure increased markedly within 1 h for most of the cases; after that, it kept on increasing slowly. There is a surface pressure increase of 0.4 mN m^{-1}/h even after 7 h for spontaneous adsorption, but after such a long period of time, the increase in surface pressure caused by the vaporization of the bulk solution cannot be neglected. This leads to complexity for the analysis of this adsorption process from the π–t curve. Considering that TCS can enter eukaryotic cells within 20 min [35], the interaction between TCS and the membrane in vivo is rather rapid and the low speed process after a long experimental time may be of little biological significance. Since the aim of the present work is to study the possible biologically meaningful interaction between TCS and phospholipids, only the initial period (100 min) of the adsorption was analyzed.

The π–t curves of adsorption under various conditions are shown in Fig. 1. The experiments shown in Fig. 1A1–A3 were performed at pH 7.4; A1 is for spontaneous adsorption, A2 for DPPC induced adsorption and A3 for DPPG induced adsorption. Fig. 1B1–B3 shows the corresponding experiments performed at pH 4.6. In each set of experiments, six different protein concentrations were used: 10 nM, 30 nM, 50 nM, 100 nM, 150 nM and 200 nM.

3.1. Induction time of protein adsorption

One of the distinct characteristics of the π–t curves shown in Fig. 1 is that there is an apparent time lag before the surface pressure starts to increase. This phenomenon has been observed for the adsorption of many proteins [27,36–39]. Cornec et al. [40] attributed this time lag to the nonlinear nature of the π–Γ relationship for the protein. Our results supported their opinion. Fig. 2 shows the π–Γ curve for TCS. We can see that the surface pressure is negligible before Γ reaches the critical value Γ_{crit}. The induction time t_0 is thought to be the time needed for the protein surface concentration to reach Γ_{crit}. So t_0 can be used as a parameter to estimate the initial

Table 1
Induction time t_0 for TCS adsorption under various conditions

	Spontaneous adsorption (min)	DPPC induced (min)	DPPG induced (min)
pH 7.4	7.0	1.0	0.5
pH 4.6	10.0	6.0	2.5

t_0 is defined as the time needed for π to reach a value of 0.2 mN/m. The bulk concentration of TCS was 100 nM.

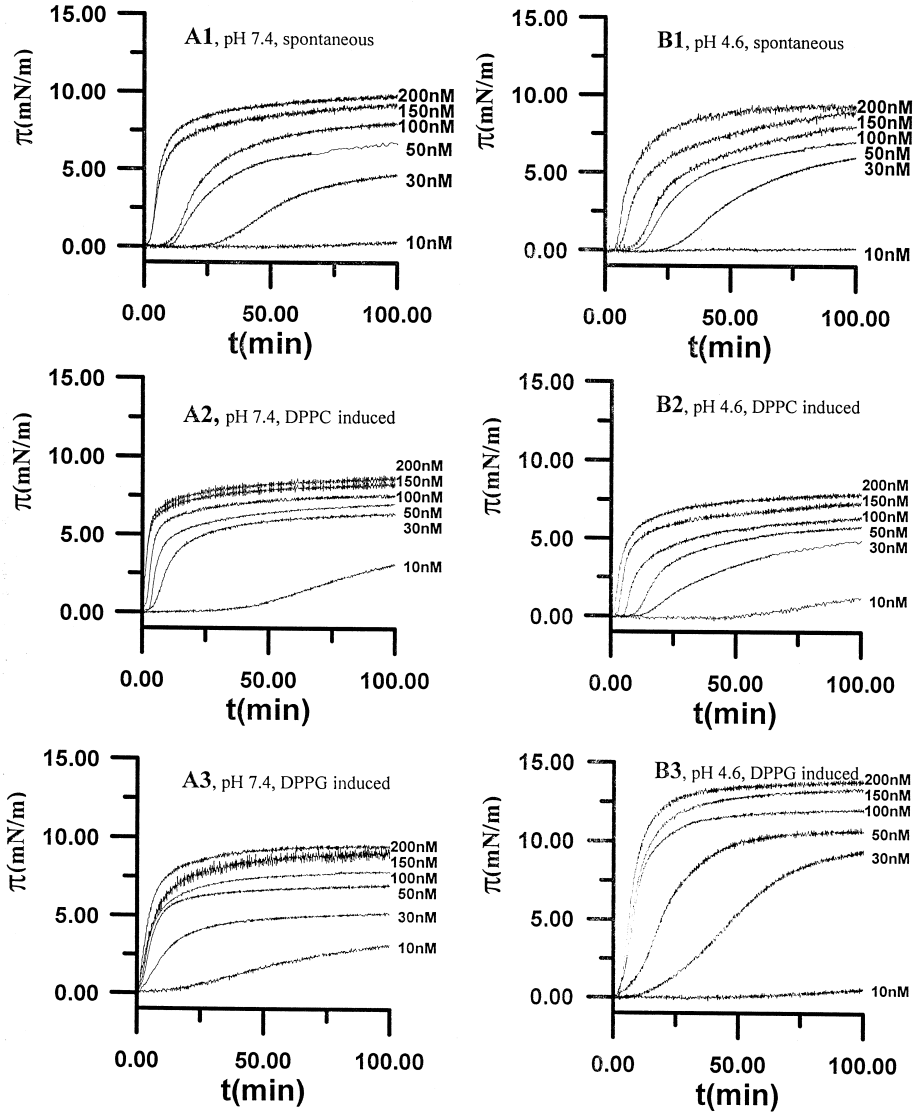

Fig. 1. The π–t curves of adsorption. A1–A3 were carried out at pH 7.4: A1 for spontaneous adsorption, A2 for DPPC induced adsorption, A3 for DPPG induced adsorption. B1–B3 are the same experiments carried out at pH 4.6. 50 mM Tris–HCl buffer was used for pH 7.4, while 50 mM sodium acetate–acetic acid (NaAc–HAc) buffer was used for pH 4.6. Six concentrations were used for each experiment: 10 nM, 30 nM, 50 nM, 100 nM, 150 nM, 200 nM.

adsorption rate. Table 1 shows the t_0 for TCS (100 nM) adsorption under various conditions. The value of t_0 is defined as the time needed for π to reach a value of 0.2 mN/m. From the results shown in Table 1, we can see that for both phospholipid (DPPC or DPPG) induced adsorptions, the values of t_0 decrease markedly compared to that for spontaneous adsorption. This result indicates a more rapid initial adsorption under phospholipid induction. Another result is that DPPG induced initial adsorption is quicker than that of DPPC. Since these two phospholipids have the same hydrophobic tail and differ only in their head group, the phospholipid head group may also contribute to adsorption induction. The electrostatic interaction between the negatively charge DPPG head group and positively charged TCS may be responsible for this difference.

3.2. Surface concentration of TCS

From the bulk concentration dependence of the TCS adsorption at the interface, the surface concen-

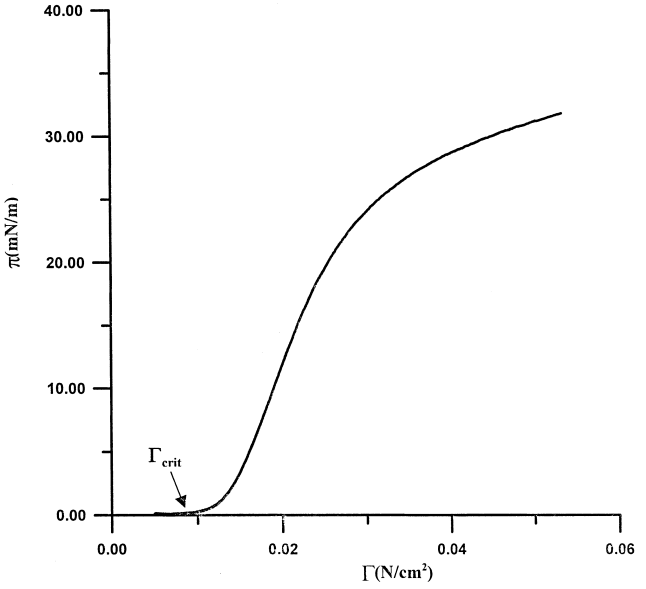

Fig. 2. π–Γ isotherm of TCS. π is the surface pressure increase caused by the excessive protein at the interface, Γ is the surface concentration of the protein. During the experiment, 180 ml of 2 nM TCS solution was added to the trough and left for 10 h before compression. The π–Γ curve was obtained by compressing the protein layer at the air–water interface with two Teflon barriers at a speed of 1 mm/min at 25 ± 0.5°C. The pH of the bulk was 7.4, and the result showed little difference when the experiment was performed at pH 4.6. Note that the unit of Γ is N/cm^2, N is the number of molecules at the interface, the value of N is unknown in this experiment.

tration (Γ) of TCS can be calculated by applying the Gibbs adsorption equation

$$d\pi = kT\Gamma\, d(\ln C)$$

where k is the Boltzmann constant, T is the experimental temperature, π is the surface pressure increase caused by protein adsorption. The values of Γ at a bulk concentration of 100 nM without and with phospholipid induction at different pH are calculated by applying the π–ln C plot, with the results shown in part I of Table 2. From the results we can find that phospholipid induction did not increase the surface concentration of TCS, but as the pH decreased from 7.4 to 4.6, the surface concentration of TCS caused by spontaneous adsorption dropped from 5.3×10^{17} mole/m^2 to 4.3×10^{17} mole/m^2, while for phospholipid (DPPC or DPPG) induced adsorption, the surface concentrations remained unchanged as the pH dropped.

It should be pointed out that the above calculated values of the surface concentration Γ may differ from the real values. Account should be made for the irreversible nature of protein adsorption before the actual concentration can be figured out [27]. However, this will not affect the final result of the relative increase or decrease in surface concentration under different conditions, so the comparison of surface concentrations under different conditions holds right. For this reason, here we still use the original form of the Gibbs equation.

For DPPG induced adsorption, despite the unaltered surface concentration at different pH, the values of the surface pressure increase (π) differs under these two pH conditions, as shown in part II of Table 2. The value of π is much higher at pH 4.6 (12.2 mN/m) than at pH 7.4 (8.0 mN/m), while the surface concentration remains the same. This phenomenon was not observed for DPPC induced adsorption.

3.3. First order equation analysis of the adsorption process

Graham et al. [28] considered the value of π determined by the amino acid residues that are packed in train configurations at the surface, so the changes in π provide a convenient way of monitoring protein penetration into the surface and conformational rearrangements of adsorbed protein molecules. The rates of these processes can be analyzed by the first order equation [41]:

$$\ln((\pi_{ss}-\pi_t)/(\pi_{ss}-\pi_0)) = -t/\tau$$

where π_{ss}, π_0 and π_t are the values of the surface pressure increase at steady-state conditions, at time $t=0$ (here $\pi_0=0$), and at any time, t, respectively, and τ is the relaxation time. This equation was also successfully used in analyzing the adsorption of some other proteins [42,43].

Fig. 3 shows the $\ln((\pi_{ss}-\pi_t)/(\pi_{ss}-\pi_0))$–$t$ curves for spontaneous adsorption and phospholipid induced adsorption; the value of the surface pressure increase after 3 h was used for the π_{ss}. The line fit of the curves showed that the process can be well divided into three steps for most of the cases except DPPG induced adsorption at pH 4.6. The first step is the induction time which has been discussed above, with no obvious surface pressure increase so the value of

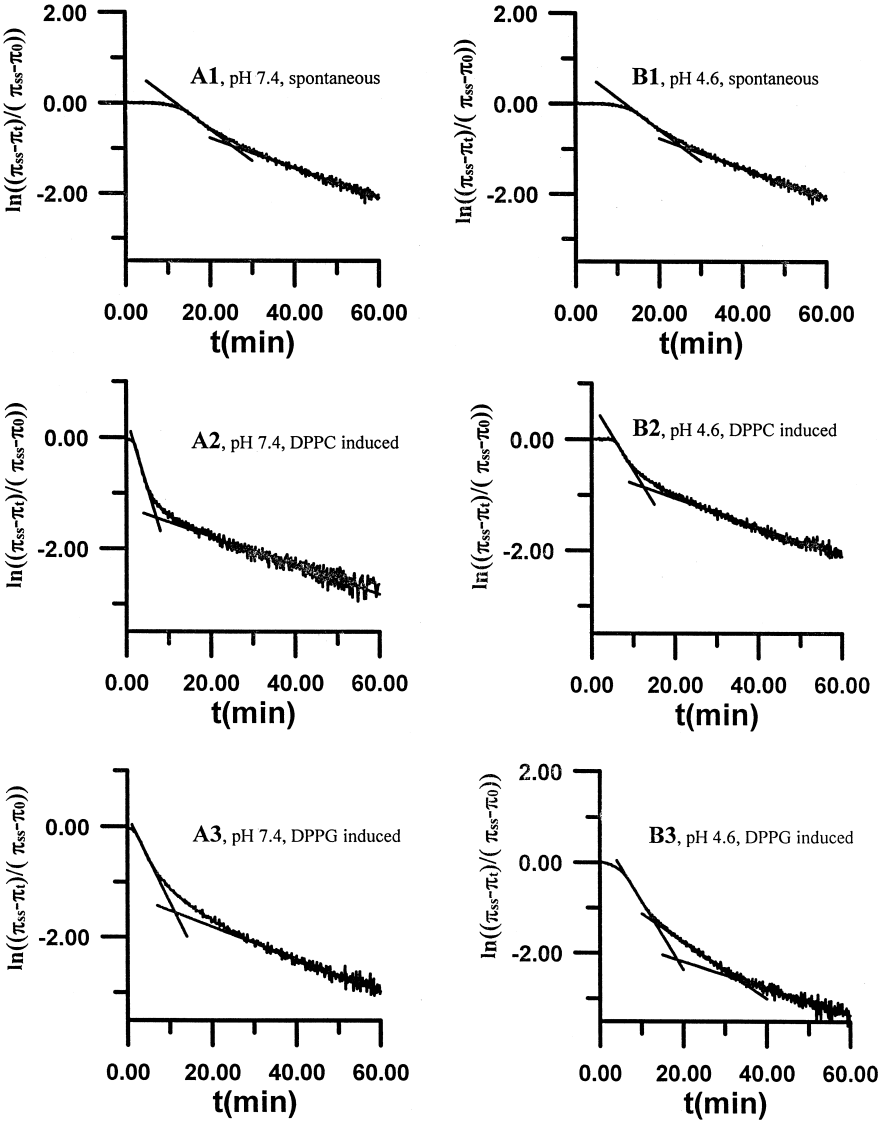

Fig. 3. The $\ln((\pi_{ss}-\pi_t)/(\pi_{ss}-\pi_0))$–$t$ curves for spontaneous adsorption and phospholipid induced adsorption. A1–A3 were carried out at pH 7.4: A1 for spontaneous adsorption, A2 for DPPC induced adsorption, A3 for DPPG induced adsorption. B1–B3 are the same experiments carried out at pH 4.6. The value of π after 3 h of adsorption was used for π_{ss}. TCS bulk concentration was 100 nM. Buffers were the same as in Fig. 1. Curves are line fitted, the slope of the line is $-1/\tau$, τ is the relaxation time.

Table 2
Surface concentration and surface pressure increase after TCS adsorption

	Spontaneous adsorption	DPPC induced	DPPG induced
(I) Surface concentration of TCS under various adsorption conditions			
pH 7.4	5.3×10^{17} molecule/m^2	3.4×10^{17} molecule/m^2	5.2×10^{17} molecule/m^2
pH 4.6	4.3×10^{17} molecule/m^2	3.4×10^{17} molecule/m^2	5.3×10^{17} molecule/m^2
(II) Adsorption caused surface pressure increase under various conditions			
pH 7.4	8.3 mN/m	7.8 mN/m	8.0 mN/m
pH 4.6	7.8 mN/m	7.0 mN/m	12.2 mN/m

For part I as well as part II the bulk concentration of TCS was 100 nM.

$\ln((\pi_{ss}-\pi_t)/(\pi_{ss}-\pi_0))$ is also around zero. The following two steps have the relaxation time τ_1 and τ_2 respectively. Graham et al. [26] considered that these two steps corresponded to the insertion of protein into the surface layer and the conformational rearrangement of the protein at the surface respectively, so τ_1 and τ_2 can be used as parameters to estimate the rate of these two processes. The values of τ_1 and τ_2 shown in Table 3 display that under phospholipid induction, τ_1 drops markedly, while τ_2 almost remains unchanged. No obvious difference between DPPC and DPPG was observed for the reduction of τ_1, excluding the possible effect of the phospholipid head group.

In the DPPG induced experiment at pH 4.6, the first order equation fit results are distinguished from the results obtained under other conditions, so the $\ln((\pi_{ss}-\pi_t)/(\pi_{ss}-\pi_0))$–$t$ curve is better divided into four steps (see the curve shown in Fig. 3B3). There seems to exist an excessive step between steps τ_1 and τ_2. We defined its relaxation time as τ'.

4. Discussion

The main purpose of this work was to study the TCS–phospholipid interaction through the difference between spontaneous TCS adsorption and phospholipid induced adsorption at the air–water interface. The introduction of phospholipid into the air–water interface influences protein adsorption at least on three sides. First, the hydrophobic tail of the phospholipid changes the hydrophobicity of the air side, which effects the adsorption of TCS. Second, there may exist electrostatic interaction between TCS and the phospholipid head group. And third, the introduced phospholipid molecules may influence the final conformation of the proteins at the air–water interface. Our results show that for most of the cases, the $\ln((\pi_{ss}-\pi_t)/(\pi_{ss}-\pi_0))$–$t$ curves for both spontaneous and lipid induced adsorption can be divided into three periods, as expected by the diffusion–penetration–rearrangement model. Therefore, this model is introduced to interpret our experimental results.

4.1. Revised model for diffusion controlled adsorption

Macritchie et al. [44] proposed the initial adsorption period to be diffusion controlled, and this opinion has been confirmed by many other studies [36,45]. In the study of Sundaram et al. [45], the induction time was shown to vary with the inverse square of the bulk concentration at low concentrations, consistent with the diffusion controlled adsorption, which should obey the equation

$$\Gamma = 2C(D{\cdot}t/3.142)^{1/2}$$

where C is the bulk protein concentration, D is the diffusion coefficient and t is the time of adsorption. Considering that t_0 is the time needed for the protein surface concentration to reach the critical value Γ_{crit}, the value of $C_2{\cdot}t_0$ should remain constant as the bulk concentration C increases. Our results showed that for spontaneous adsorption at low protein concentrations, the induction times (t_0) are 210 min, 28 min and 11 min for a protein concentration of 10 nM, 30 nM and 50 nM at pH 7.4 respectively (while the induction time is 200 min, 26 min, 11 min for pH 4.6). The values of $C^2{\cdot}t_0$ are about the same when the protein concentration increases, which supports the diffusion controlled mechanism.

The introduction of phospholipid in the protein adsorption process reduced the values of t_0 significantly, but the values of both protein concentration and diffusion coefficient were not changed. Fig. 4 shows schematically our explanation for t_0 reduction.

The diffusion controlled model assumed that every collision of a protein molecule with the surface leads

Table 3
Relaxation times τ_1 and τ_2 for adsorption under various conditions

	pH 7.4			pH 4.6		
	Spontaneous	PC induced	PG induced	Spontaneous	PC induced	PG induced
τ_1 (min)	14	4	6	15	8	7
τ_2 (min)	30	34	30	35	38	34

The bulk concentration of TCS was 100 nM.

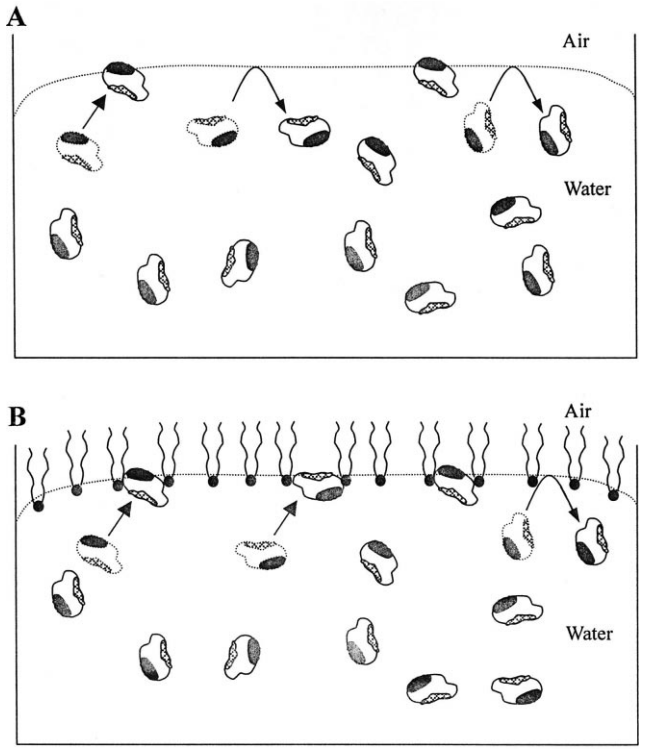

Fig. 4. A revised model for diffusion controlled adsorption. (A) Spontaneous adsorption; (B) phospholipid induced adsorption. Proteins are randomly oriented in the bulk. Spontaneous adsorption happens only when proteins with appropriate orientation (in this sketch map, orientation with the black part upside) collide with the surface. Proteins with other orientations are rejected back. When phospholipid was introduced into this system (B), it can help to stabilize the proteins at the interface, so adsorption also happens when proteins with the shaded part upside collide with the surface.

to adsorption. In our revised model, however, adsorption happens only when protein molecules with appropriate orientation collide with the surface. It is likely that this appropriate orientation is the orientation with a hydrophobic part facing upside, so it stays stable at the air–water interface while other orientations do not. Protein molecules are randomly oriented in the bulk; those with inappropriate orientations are rejected back when colliding with the surface. The presence of phospholipid at the air–water interface leads to protein–lipid interaction that may help to stabilize the protein at the interface. So some of the originally inappropriately orientated proteins are now not rejected back when they collide with the interface because phospholipid molecules help them to stay at the interface. In this way the effective collision between TCS and the interface increases, and the initial adsorption rate is enhanced. We can also see that not only the hydrophobic tail of a phospholipid can help to stabilize the protein at the interface, but the head group of a phospholipid also contributes, for although DPPC and DPPG both decrease the induction time, the effect of DPPG is more distinct than that of DPPC. So there are both hydrophobic interaction and electric interaction between TCS and phospholipids, and these interactions help to stabilize TCS at the air–water interface.

Since the three-dimensional structure of TCS has now been resolved to a high resolution [17,18], the hydrophobic and hydrophilic distribution on the molecular surface was computed using GRASP [46]. The result showed clearly that the hydrophobic parts were asymmetrically distributed on the molecular surface. The hydrophobic-rich side is 30.0% occupied by hydrophobic surfaces, while its opposite side is hydrophilic-rich, and is only 12.1% occupied by hydrophobic surfaces. The result is shown in Fig. 5. So the protein molecule can be well divided into a hydrophobic side and a hydrophilic side. The above mentioned appropriate orientation may correspond to this hydrophobic side facing upside, while orientations with the hydrophilic side facing upside were rejected back when they collided with the interface. When phospholipid was introduced into the system, some of the intermediate orientations were also accepted as the appropriate orientation.

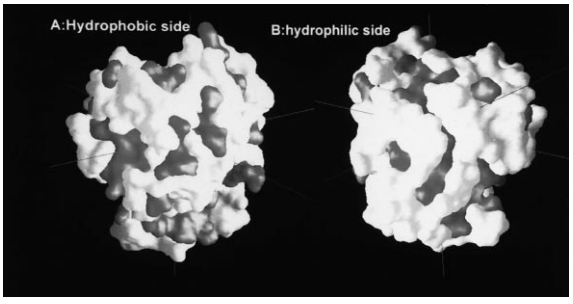

Fig. 5. The hydrophobic and hydrophilic sides of TCS. The hydrophobic surface (gray) and the hydrophilic surface (white) were calculated using GRASP V1.3. The percentage of hydrophobic surface was 30.0% for the hydrophobic side and 12.1% for the hydrophilic side.

4.2. Surface concentration and surface pressure increase

As the bulk pH decreased, the surface concentration of TCS for spontaneous adsorption dropped, as shown in part I of Table 2. TCS is a basic protein with an isoelectric point of 9.4, so the net positive charge on its surface increases as the pH drops from 7.4 to 4.6, and the electrostatic repulsion between molecules increases as a result. This may be responsible for the result that the surface concentration of TCS was lower at pH 4.6 than at pH 7.4. However, when phospholipid is introduced into the interface, the protein may be unfolded by the hydrophobic interaction and the interior residue exposed in this condition, some of the charged residues are replaced by non-charged hydrophobic residues, and the effects of electrostatic repulsion were weakened. So under different pH conditions, the surface concentrations for both DPPC and DPPG induced adsorption are about the same. This result suggests on the other hand that there is strong hydrophobic interaction between TCS and the phospholipid hydrophobic tail. This is also consistent with our previous result that TCS can penetrate into the phospholipid monolayer under appropriate conditions [24].

We also noticed that for DPPG induced adsorption, the surface pressure increase is significantly higher at pH 4.6 (12.2 mN/m) than at pH 7.4 (8.0 mN/m), while the surface concentrations of TCS are comparable under these two pH conditions. This is possibly caused by the conformational change of TCS under DPPG induction at low pH conditions, which leads to a larger surface area occupied by one protein molecule. This possible conformational change is discussed further below.

4.3. Dynamic analysis of TCS adsorption

Analysis of the π–t curves by applying the first order equation revealed that the adsorption process can be divided into three steps for most of the cases. It is proposed by Graham and Phillips [26] and widely accepted that step 2 corresponds to the penetration of a protein molecule into the surface layer, while step 3 is mainly caused by the conformational rearrangement of proteins at the surface. The rates of these two steps can be described by the relaxation times τ_1 and τ_2. Our results showed that in a phospholipid induced adsorption experiment, the relaxation time τ_1 drops compared to spontaneous adsorption, but relaxation time τ_2 remains unchanged. According to the model of Graham and Phillips, the penetration of proteins into the surface layer is accelerated under phospholipid induction, while the conformational rearrangement of proteins is not influenced. The speed up of the τ_1 step (penetration step) is thought to be due to the interaction of the hydrophobic groups on the protein surface with the hydrophobic chain of the phospholipid. The hydrophobic tail of the phospholipid provides a more hydrophobic environment compared to the air, which leads to the lowering of the energy barrier for the protein insertion process, thus the insertion of TCS is accelerated. The rate of the τ_2 step (conformational rearrangement step) is thought to be mainly determined by the stability of the protein structure, rigid structure results in a longer relaxation time τ_2, so the speed of this process is not necessarily altered by the phospholipid induction.

An excessive τ' step was observed for DPPG induced adsorption at pH 4.6. This step was not observed for spontaneous adsorption and DPPC induced adsorption, nor is it notable for DPPG induced adsorption at pH 7.4. Considering our previous work [23], the negatively charged DPPG membrane may induce a conformational change of TCS under acidic conditions. This conformational change was unique for the above condition; it is not observed for DPPG at neutral pH, or for DPPC under any pH condition. So it is possible that this step corresponds to this DPPG induced conformational intermediate of TCS, and this conformational change may lead to the increase in the area occupied by TCS molecules at the surface, hence the surface pressure increase for DPPG induced adsorption is higher at pH 4.6 than at pH 7.4, whereas the surface concentration of protein is not enhanced under this condition.

4.4. Conclusion

From the difference between TCS adsorption with and without phospholipid induction the conclusion was drawn that this protein interacts strongly with the hydrophobic tail of the phospholipid, for both

the induction time t_0 and the insertion relaxation time τ_1 were reduced under phospholipid induction. There is also a certain electric interaction between TCS and the phospholipid head group, because the induction time is further reduced for DPPG compared with that of DPPC. This is understandable because TCS is a basic protein with p*I* 9.4, so its positively charged residues at the surface may be attracted by the negatively charged head group of DPPG. A revised diffusion controlled model is also proposed in this work to explain the reduction of the induction time.

Analysis of the π–t curves using the first order equation revealed a possible additional conformational change for DPPG induced adsorption under low pH condition, but not for DPPC induced adsorption. This conformational change enlarges the area occupied by the TCS molecule at the interface which results in a higher surface pressure increase. The additional conformational change is consistent with our previous result that there is a conformational intermediate when TCS penetrates into the negatively charged phospholipid under low pH condition [23].

Acknowledgements

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