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# Molecular recognition of concanavalin A on mannoside diacetylene lipid monolayer at the air–water interface

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**Abstract**

The interaction of *p*-10,12-pentacosadiyne-1-*n*-phenylamide α-D-mannopyranoside (MPDA) with protein concanavalin A (Con A) was studied at the air/water interface. The expansion of molecular area of PDA (10,12-pentacosadiynoic acid)/MPDA mixed monolayer after injection of Con A in subphase shows strong interaction between Con A and the monolayer. The maximum expansion of molecular area decreases as the molar ratio of MPDA increases due to the steric hindrance effect. By using enzyme mannosidase to cut-off the mannoside headgroup of MPDA, expansion of molecular area was greatly reduced, indicating that the binding of Con A is specific to the mannoside headgroup. The kinetics of the binding fits to the first order bimolecular reaction model. Fluorescence quenching of fluorescein isothiocyanate labeled Con A after injection into the subphase gives a direct proof of the molecular recognition. 

*Keywords:* Concanavalin A; Mannoside diacetylene lipid; Langmuir monolayer; Molecular recognition

## 1. Introduction

Bacterial toxins are associated with many diseases in humans and animals [1]. Binding of toxins to carbohydrate receptors on human cells represents the initial step in the invasion and infection of many bacterial toxins [2]. The study of molecular recognition processes between the receptors and ligands is an important step toward the understanding of many biological phenomena. Langmuir monolayer at the air/water interface has been demonstrated to be useful in cell surface modeling. For the recognition process at the air/water interface, the interfacial behavior of the Langmuir monolayer can be used to probe the recognition process. Different types of transducers can also be used to detect the molecular recognition processes between a monolayer and the ligand in solution, such as surface plasmon resonance spectroscopy [3,4] and quartz crystal microbalance [5]. Optical signals, such as absorption and fluorescence, are another class of sensitive transducers.

The use of a stable matrix lipid to entrap the receptor molecule into the monolayer is an important condition for the study of the recognition process. Diacetylene lipid monolayer can be polymerized by UV irradiation, the polymerization process can be easily monitored by absorption spectra due to the strong absorption in the visible range [6,7]. The polymerized diacetylene network could enhance the

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stability of mixed monolayer with the receptor molecule entrapped into it.

Concanavalin A (Con A) was chosen as a model ligand for our molecular recognition study. Con A is the lectin of the jack bean (*Canavalia ensiformis*). This protein is a widely used probe for studies of the cell surface and of the regulation of cell metabolism and cell division. It can specifically bind to saccharide-containing receptors, such as D-glucose or D-mannose containing molecules. Con A is a subunit protein, with a protomer molecular weight of 25 500 Da. The subunits predominantly form dimers below pH 6 and tetramers and higher aggregates above pH 7. Each protomer has a binding site for $Mn^{2+}$, $Ca^{2+}$ and saccharide. The binding of saccharide requires the present of both metal ions [8].

The model receptor, *p*-10,12-pentacosadiyne-1-*n*-phenylamide α-D-mannopyranoside (MPDA) was synthesized from 10,12-pentacosadiynoic acid (PDA). Surface chemistry studies on mixed PDA/MPDA monolayers show that these monolayers are miscible at the air/water interface for a wide range of PDA/MPDA ratios, surface pressures and subphase pHs [9].

The molecular recognition of PDA/MPDA monolayer with Con A at the air/water interface was studied by both Langmuir and fluorescent-labeling techniques.

## 2. Materials and methods

PDA was purchased from Farchan Laboratories (Gainsville, FL). The compound was purified by recrystallization with petroleum ether (36–60°C). MPDA was synthesized from PDA [9].

All the organic solvents used in this study are HPLC grade (Fisher Scientific, Pittsburgh, PA). Chloroform was purified by distillation, and the component between 61.1 and ~61.3°C was collected and used. Solutions of 1.0 mM of pure PDA, MPDA and their mixtures in chloroform and methanol solvent (5:1 in molar ratio) were prepared. The purpose of methanol is to increase the solubility of MPDA.

Con A, fluorescein isothiocyanate (FITC) labeled Con A and β-mannosidase were purchased from Sigma (St. Louis, MO). FITC-Con A contains approximately 3.6 mol FITC per mol of Con A (tetramer). One unit of β-mannosidase can hydrolyze 1 μmol of *p*-nitrophenyl-β-D-mannopyranoside to *p*-nitrophenol and D-mannopyranoside per minute at pH 4.0 and 25°C. These proteins were used without further purification.

The water used for monolayer study was purified by a Modulab 2020 water purification system (Continental Water Systems, San Antonio, TX). The water has a resistance of 18 MΩ-cm and a surface tension of 72.6 mN $m^{-1}$ at 20°C. The surface tension was measured by Digital-Tensiometer K10 (Krüss Wissenschaftliche, Hamburg, Germany). Cadmium chloride was certified ACS grade (Fisher Scientific, Pittsburgh, PA).

Monolayer kinetics were measured on a KSV mini-trough system under room temperature (22.0 ± 0.5°C) with humidity of 40 ± 5%. The trough has an area of 225 $cm^2$. The pressure sensor has a resolution of 0.02 mN $m^{-1}$. For each experiment, 40 μl of 1.0 mM sample were spread on the water surface, waiting 10 min for solvent evaporation before compression. The barrier was compressed at a speed of 2.5 $Å^2$ $molecule^{-1}$ $min^{-1}$.

The fluorescence spectrum of the monolayer at the air/water interface was measured by an optical fiber detector which was connected to a SPEX Fluorolog II fluorospectrometer.

All the surface chemistry experiments were performed in a class 1000 clean room.

## 3. Results

PDA/MPDA mixed monolayers with different molar ratio of MPDA were spread on a subphase containing $10^{-3}$ M $CdCl_2$, $10^{-4}$ M $MnCl_2$ and $10^{-4}$ M $CaCl_2$ (pH 4.4). The monolayers were compressed and maintained at the surface pressure of 10 mN $m^{-1}$, then Con A solution was injected into the subphase by a long needle syringe. To avoid disturbing the monolayer, the needle was inserted into the subphase from outside of the barrier. To ensure an equal distribution of Con A in the subphase, it was injected in multiple places of subphase. The final concentration of Con A in the subphase is 0.01 mg $ml^{-1}$, which has a total amount of 0.015 μmol tetramer in subphase. At pH 4.4, Con A is in the form of dimer. The total amount of Con A dimer in the

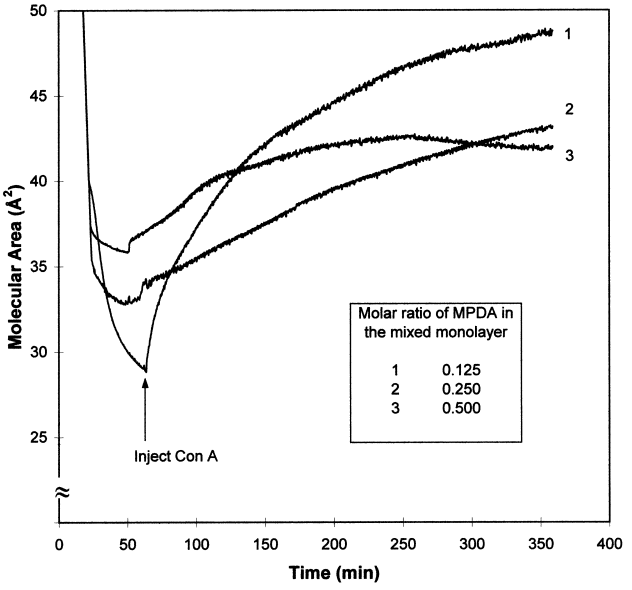


Fig. 1. Interaction of Con A with PDA/MPDA mixed monolayers at the air/water interface: effect of MPDA molar ratio. PDA:MPDA = 7:1 (1), 3:1 (2), 1:1 (3), respectively. Surface pressure was held at 10 mN/m. Subphase, $10^{-3}$ M $CdCl_2$, $10^{-4}$ M $MnCl_2$ and $10^{-4}$ M $CaCl_2$, pH 4.4; Con A, 0.01 mg $ml^{-1}$.

subphase is 0.03 μmol, which is in excess of the amount of MPDA in the monolayer (about 0.005–0.02 μmol, depends on the molar ratio of MPDA in the monolayer). Immediately after the injection, there was a gradual increase in the area of the monolayer for a few hours (Fig. 1). PDA/MPDA mixtures at different molar ratios show different expansions of the molecular area. This indicates that there are strong interactions between Con A and the lipid monolayer. The monolayer with a MPDA molar ratio of 0.125 shows the maximum expansion of molecular area. The expansion of molecular area decreases with the increasing of MPDA molar ratio.

The following experiments were done to reveal the type of interaction between Con A and the monolayer. After spreading the same monolayer (0.125 MPDA molar ratio) on the same subphase and maintained at a surface pressure of 10 mN $m^{-1}$, enzyme β-mannosidase was injected into the subphase, to make the subphase contain $4\times10^{-4}$ U $ml^{-1}$ mannosidase. It has been pointed out that one unit of β-mannosidase can hydrolyze 1 μmol of *p*-nitrophenyl-β-D-mannopyranoside per minute at pH 4.0 and 25°C. The amount of MPDA on the surface is 0.005 μmol, while the amount of mannosidase in the subphase is about 0.04 U. Thus, the MPDA/mannosidase ratio in the system is 0.125 μmol $U^{-1}$. Under ideal conditions, the mannopyranoside group of the monolayer will be cut-off in a minute. Considering the slightly different pH (4.4 instead of 4.0), low temperature (22°C) and two-dimension reaction, the actual reaction speed will be slower. Nevertheless, most of the mannoside headgroups should be cut-off 1 h after the injection of mannosidase. During this period, the area of the monolayer shows a slight decrease (1.3 $Å^2$), which could be attributed to the removal of mannosidase group of MPDA. Then, the same amount of Con A was injected into the subphase as above, and the molecular area expansion of the monolayer was very small (Fig. 2). This result shows that the Con A is specifically bound to the mannoside group of MPDA.

Further evidence of the binding of Con A with the lipid monolayer came from the fluorescence spectroscopic experiments at the air/water interface. FITC labeled Con A (FITC-Con A) was used instead of Con A. The mixed monolayer was the same and the surface pressure was kept constant at 10 mN $m^{-1}$.

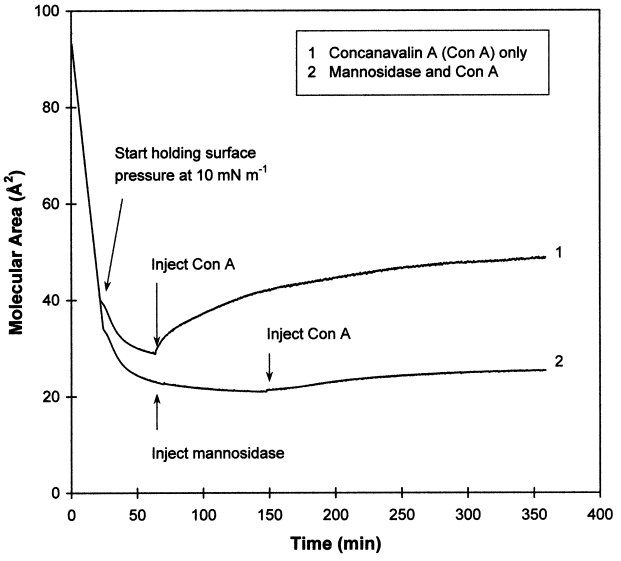


Fig. 2. Interaction of Con A with PDA/MPDA mixed monolayers at the air/water interface: effect of mannosidase. PDA:MPDA = 7:1 (0.125 MPDA molar ratio). Surface pressure was held at 10 mN $m^{-1}$. Subphase, $10^{-3}$ M $CdCl_2$, $10^{-4}$ M $MnCl_2$ and $10^{-4}$ M $CaCl_2$, pH 4.4; Con A, 0.01 mg $ml^{-1}$; mannosidase, $4\times10^{-4}$ U $ml^{-1}$.

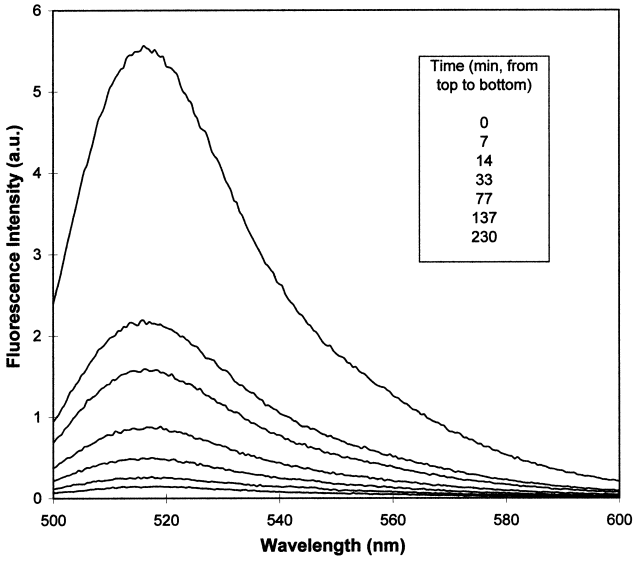


Fig. 3. Fluorescence spectra of FITC-Con A: interaction with PDA/MPDA (7:1 in molar ratio) mixed monolayer at the air/water interface. FITC-Con A was injected into subphase to 0.01 mg $ml^{-1}$ at time 0 min. Emission scans start from 500 nm to avoid the peak of excitation light at 470 nm. Surface pressure, 10 mN $m^{-1}$; subphase, $10^{-3}$ M Tris-HCl, $10^{-4}$ M $MnCl_2$ and $10^{-4}$ M $CaCl_2$, pH 7.5.

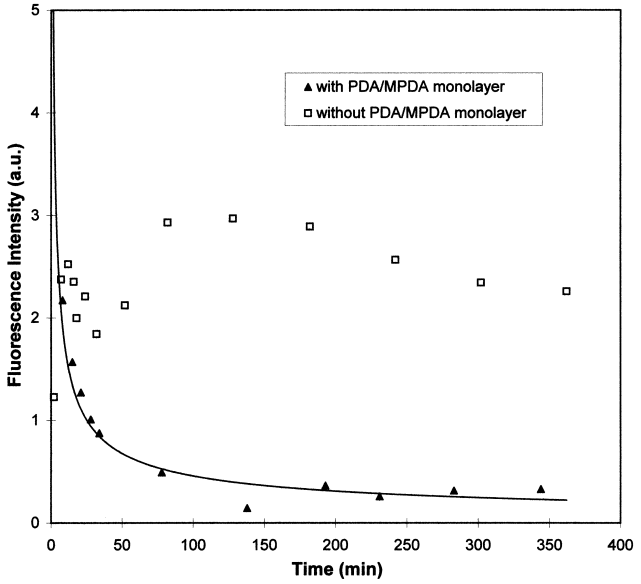


Fig. 4. Kinetic of FITC-Con A interaction with and without PDA/MPDA mixed monolayer at the air/water interface. $\lambda_{em}$ = 517 nm; $\lambda_{exc}$ = 470 nm; PDA:MPDA = 7:1 (0.125 MPDA molar ratio); FITC-Con A, 0.01 mg $ml^{-1}$.

The subphase contained $10^{-2}$ mg $ml^{-1}$ FITC-Con A and $10^{-3}$ M Tris-HCl, $10^{-4}$ M $MnCl_2$ and $10^{-4}$ M $CaCl_2$ (pH 7.5, to keep the FITC-Con A in tetramer format and maintain the fluorescence activity). The fluorescence spectrum of FITC-Con A was measured at an excitation wavelength of 470 nm. The maximum fluorescence intensity was observed at 517 nm. Its intensity kept decreasing for several hours after the injection (Fig. 3). For the same experiment without any monolayer on the surface, the fluorescence intensity remained constant (Fig. 4). In both experiments, no peak shift was observed.

## 4. Discussion

The expansion of the molecular area of the PDA/MPDA monolayer upon the injection of Con A is a strong line of evidence of interactions between Con A and PDA/MPDA. According to the X-ray diffraction studies [8], the Con A protomer has a diameter of about 40 Å, which is five times bigger than the lipid molecules. At pH 4.4, Con A forms a dimer, which has an even bigger size compared to the protomer. Thus, the expansion of the molecular area of

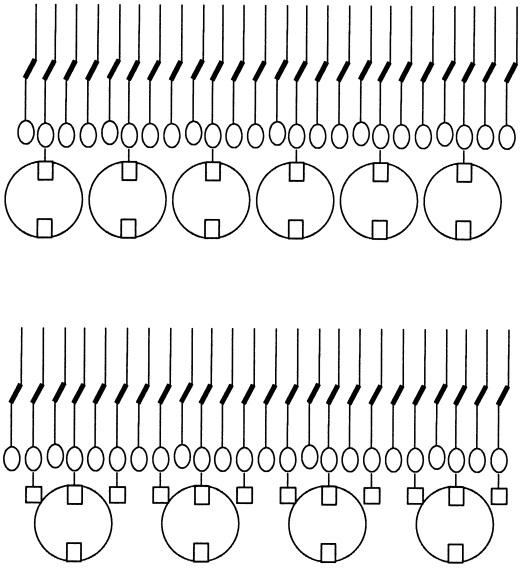

Fig. 5. Steric hindrance effect of Con A binding to PDA/MPDA monolayer. Upper panel: when MPDA molar ratio is low, Con A easy binds to mannoside headgroup. Lower panel: when MPDA molar ratio is high, fewer Con A binds to the monolayer because of the lack of lateral space on the side of mannoside headgroup.

the monolayer is due to the large lateral space required by Con A after the binding.

Fig. 1 shows that the maximum area expansion of the monolayer decreases almost linearly with an increase in the MPDA molar ratio. This indicates that Con A is specifically bound to the mannoside group of MPDA. If this is the case, it can be explained by steric hindrance effect as shown in Fig. 5. Due to the large size of Con A, the binding requires a large space around the side of the mannoside head group. The more MPDA molecules in the monolayer, the less free space between the side of mannoside headgroup, and the binding becomes more difficult. If the binding of Con A to the monolayer were non-specific, it would be difficult to explain the relationship between the MPDA ratio and maximum expansion of molecular area.

The above explanation is strongly supported by the mannosidase experiment. After the mannoside groups were cut-off from the MPDA molecules, the maximum expansion of molecular area upon injection of Con A decreased from 19.7 to 3.9 Å$^2$. This can only be explained by assuming a specific interaction between Con A and the mannoside group of MPDA.

A reasonable model to describe the binding of Con A to the monolayer is a reversible bimolecular reaction [10]

$$C + M \underset{k_r}{\overset{k_f}{\rightleftharpoons}} CM \quad (1)$$

where $C$, $M$, and $CM$ represent free Con A, unbound MPDA and Con A/MPDA complex after binding, respectively, $k_f$ and $k_r$ are forward and reverse reaction rate constants, respectively. According to first-order kinetics, the rate of formation of $CM$ is given by

$$d[CM]/dt = k_f[C][M] - k_r[CM] \quad (2)$$

where $[C]$ is the concentration of Con A in subphase,

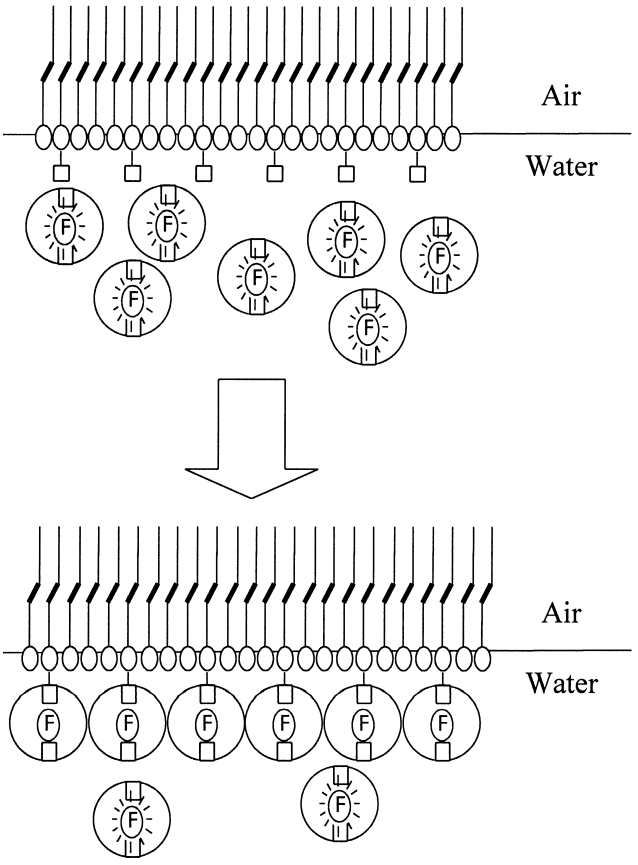


Fig. 6. Fluorescence quenching effect of FITC-labeled Con A after binding to the PDA/MPDA monolayer.

$[M]$ and $[CM]$ are the surface density of MPDA and Con A/MPDA complex. Since the number of Con A in subphase is much larger than the number of MPDA on the surface, we have

$$[C] \approx [C]_0 \quad (3)$$

$$[M] = [M]_0 - [CM] \quad (4)$$

where $[C]_0$ is the initial concentration of Con A in subphase and $[M]_0$ is the initial surface density of MPDA. From Eqs. 2–4, we obtain the time-

Table 1
Kinetic constant of Con A bound to MPDA obtained from non-linear regressions

| Molar ratio of MPDA | 0.125 | 0.250 | 0.500 | 0.125 (mannosidase) |
|---|---|---|---|---|
| $\Delta A_e$ (Å$^2$ molecule$^{-1}$) | 19.6 | 15.8 | 6.1 | 5.0 |
| $t_{1/2}$ (min) | 51 | 221 | 47 | 67 |
| Correlation coefficient | 0.982 | 0.981 | 0.967 | 0.996 |

dependent increase in the formation of the *CM* complex

$$[CM](t) = [CM]_e(1-\exp(-(\ln 2)t/t_{1/2})) \quad (5)$$

where $[CM]_e$ is the surface density of the complex at equilibrium, $t_{1/2}$ is the time constant when $[CM] = [CM_e/2$. Both $[CM]_e$ and $t_{1/2}$ are defined functions of $k_f$, $k_r$, $[C]_0$ and $[M]_0$. If we assume that the molecular area expansion of the monolayer upon the injection of Con A is linearly proportional to the surface density of Con A/MPDA complex, then we have

$$\Delta A(t) = \Delta A_e\ (1-\exp(-(\ln 2)t/t_{1/2})) \quad (6)$$

where $\Delta A$ is the molecular area expansion of the monolayer upon the injection of Con A, and $\Delta A_e$ is the molecular area expansion of the monolayer at equilibrium. By using a non-linear regression program (SigmaPlot for Windows, from Jandel Corporation), we calculated $\Delta A_e$ and $t_{1/2}$ from our data, which are shown in Table 1. The correlation coefficient of the regressions is close to 1, which means our data is fit to the model well. This again confirms the specific binding between Con A and the mannoside group.

In the fluorescence spectroscopy experiment, the decrease in the fluorescence intensity is explained by the binding of FITC-Con A with the lipid monolayer. The binding of Con A to the lipid monolayer will result in a higher concentration of the protein on the surface. Consequently, the probability of energy transfers between the free Con A molecules and the aggregated Con A molecules increases. Part of the excitation light could be absorbed by the aggregated Con A domains at the air/water interface. The aggregated Con A should have a very low fluorescent efficiency compared to the unbound Con A molecules, which means that most of the excitation energy would be converted to heat (Fig. 6). The decrease of fluorescence intensity reaches equilibrium in about 5 h, which is comparable to the time scale of molecular area expansion of the monolayer after the injection of Con A. A similar non-linear regression as above for the fluorescence intensity was performed. The results show a poor correlation between the data and the kinetic model, which can be explained by the fact that fluorescence quenching has a non-linear relationship with the surface density of Con A/MPDA complex.

## 5. Conclusion

Surface chemistry and fluorescence spectroscopy studies show that Con A can specifically bind to the mannoside headgroup of PDA/MPDA monolayer. The amount of Con A bound to the monolayer depends on the molar ratio of MPDA, which is explained by the steric hindrance effect. The binding is significant, while the molar ratio of MPDA is less than 0.5. These results present a Langmuir monolayer approach of the molecular recognition studies, which could have substantial applications in biosensor development.

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