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Specific recognition and two-dimensional organization of molecules at the air–water interface

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Abstract : Complementary hydrogen bonding acts efficiently at the air waterinterfacefor specific binding of aqueous guests. Thus, single-component and mixed monolayers of double-chain peptide amphiphiles displays selective incorporation of water-soluble dipeptides via hydrophobic and hydrogen-bonding interaction into the specific cavities created at the polar peptide region of monolayers. An "induced fit" mechanism is conceivable for the generation of receptor sites in mixed monolayers. The hydrogen-bonding interaction is also applicable to the preparation of molecularly precise patterns on water. The molecular arrangement in guanidinium and melamine monolayers is controlled through binding with polycarboxylates and barbituric acid, respectively. A multi-functional guest like FAD can induce molecular patterns. Implications of these results are discussed.

INTRODUCTION

Hydrogen bonding is most directional among the secondary valence forces that control the biological molecular process and maintain the precise structure of biological macromolecules. Therefore, the host-guest interaction by specific hydrogen bonding has been a central issue in molecular recognition research, and a large number of artificial molecular hosts have been synthesized for this purpose. Hydrogen bonding involved in these interactions is most effective in aprotic organic solvents, because of strong competition with water in the aqueous environment. Recently we demonstrated that efficient molecular recognition is possible by complementary hydrogen bonding in spite of bulk water at the air-water interface.(ref. 1) This unexpected finding is explicable at least partially by taking into account the unique microenvironment of the interface.(ref. 2)

We discuss in the present article an extension of these findings to specific recognition of aqueous peptides and the formation of molecular patterns as a result of hydrogen bond-mediated host-guest binding at the air-water interface.

SPECIFIC RECOGNITION OF DIPEPTIDES DISSOLVED IN WATER

Hydrogen bonds play a most important role in defining precise three-dimensional structures of proteins and their supramolecular complexes. The nature and strength of the hydrogen bond in amino acids and peptides have been widely studied in artificial systems. Most of these

investigations were done in isotropic environments; however, many biological interactions occur at the macromolecular interface, especially at the surface of biomembranes. Organized molecular monolayers and bilayers are especially useful as mimics of these interactions occurring at the interface, because they can provide uniquely oriented surface environments.

Monolayer of single-chain peptide amphiphiles

As a first attempt for this purpose, we investigated the monolayer behavior of some monoalkyl oligoglycine amphiphiles.(ref. 3) They formed stable crystalline monolayers. Figure 1 displays a conceivable model for monolayers of these amphiphiles by using $C_{18}Gly_3OEt$ as a typical example. The packed alkyl chains on the water surface help their Gly_3 moieties form hydrogen bonds with each other, and the strengths of these hydrogen bonds can be increased with increasing numbers of the glycine residue in an amphiphile. Larger molecular areas than expected from their molecular cross sections imply that the alkyl chains in the monolayer are packed in a tilted arrangement and that the intermolecular hydrogen bonding is longer and weaker at the interface than those in crystal.

Higashi *et al.* (ref. 4) studied the structure of the poly(L-glutamic acid) moiety appended to double alkyl chains at the air/water interface, and found that a stable β-structure was formed in the condensed phase when the peptide moiety (degree of polymerization ~ 40) was aligned in monolayers. Conventional poly(L-glutamic acid) displays a conformational transition between α-helix and random coil. It is clear that spatial confinement and chain alignment at the interface induced the β-structure rather than the conventional α-helix. Our study provides an additional example of a peptide conformation at the interface. The oligoglycine moiety in the monolayer forms packed hydrogen bonding structures similar to that of polyglycine II as evidenced by the common IR features. The maintenance of a specific conformation for short peptides (Gly_3 to Gly_5) is interesting, since it is not usually probable in bulk solution. This must be caused by conformational fixation through intermolecular hydrogen bonding. On the other hand, the conformational fixation may reflect the unique nature of the interface. If this contribution is significant, short peptide chains on the biological molecular surface may assume specific (functionally important) conformations that are not probable at non-interfacial sites.

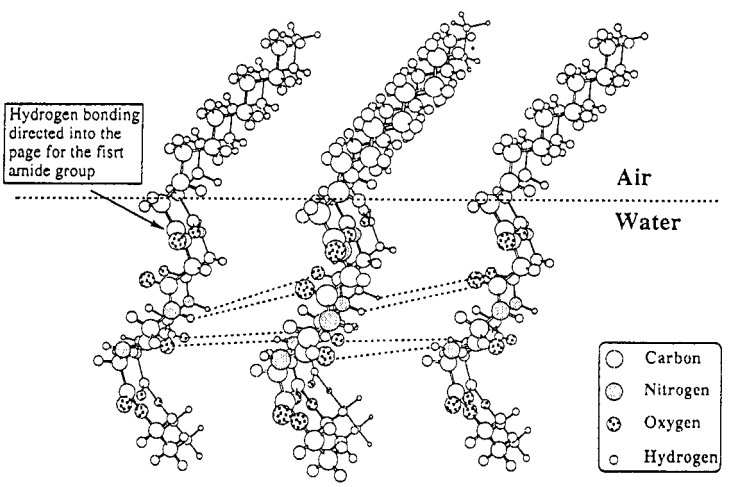


Fig. 1 A schematic representation of molecular packing and the pattern of hydrogen bonding for the $C_{18}Gly_3OEt$ monolayer. Dotted lines show hydrogen bonds.

Peptide recognition by monolayers of double-chain oligoglycine amphiphiles

The single-chain peptide monolayers did not show any binding capability towards aqueous peptides. Apparently, strong intermolecular hydrogen bonding destroyed additional interaction with guest molecules. This situation may be remedied by weakening the intermolecular amide-amide interaction. In fact, monolayers of some double-chain peptide amphiphiles showed superior receptor properties towards oligopeptides dissolved in the aqueous subphase.(ref. 5) In particular, the binding property of $2C_{18}BGly_2NH_2$ monolayer was examined for a large variety of dipeptides. The amount of guest dipeptides that accompanied the LB film of $2C_{18}BGly_2NH_2$ was determined on the basis of the C/N ratio in the XPS analysis.

Figure 2 compares the extent of dipeptide incorporation from their 0.01 M solutions. GlyX dipeptides (X: Leu, Phe, Pro, and Ala) are specifically bound, but X'Gly (X': Leu and Phe) are not. The other dipeptides (HisLeu, AlaAla, and LeuLeu) are not detectably bound under the same experimental conditions. Plausible models of incorporation of aqueous GlyPhe to a receptor site of $2C_{18}BGly_2NH_2$ monolayer are depicted in Fig. 3. An optimization of the amphiphile conformation suggests that the two alkyl chains are placed perpendicular to the benzene plane (rather than parallel to it), as shown in this figure. A guest dipeptide (GlyPhe) is inserted into the receptor site from the C-terminal with the hydrophobic side chain of the Phe residue being laid between the benzene planes of the host molecule. The two terminal functions and the amide groups of the guest peptide apparently form two pairs of antiparallel hydrogen bonds with diglycyl units of the host molecule. The hydrophobic side chain of the second amino acid residue can be stably accommodated in this scheme, consistent with the observed dipeptide specificity. The cooperative effect of hydrophobic packing and hydrogen bonding would enhance substrate binding. The hydrophobic side chain of PheGly dipeptide cannot be accommodated snugly in this binding model.

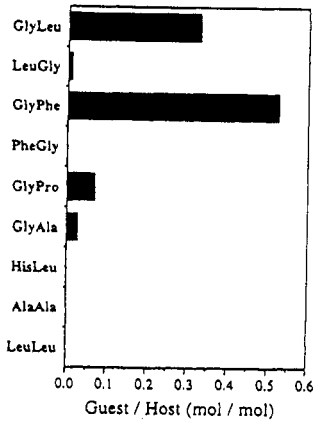


Fig. 2 Binding ratio (guest/host) of different aqueous dipeptides by $2C_{18}BGly_2NH_2$ monolayer.

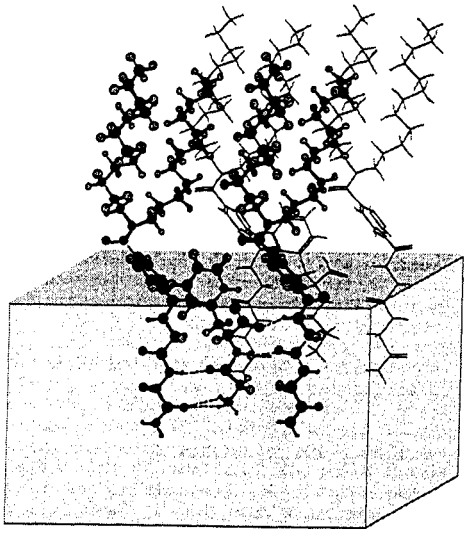


Fig. 3 A conceivable pattern of hydrogen bonding interaction between $2C_{18}BGly_2NH_2$ monolayer and GlyPhe dipeptide. The guest peptide of GlyPhe is inserted into the monolayer from C-terminal.

Peptide recognition by mixed peptide monolayers

Efficient binding of aqueous GlyX dipeptides by the single-component oligoglycine monolayer was promoted by three key factors: (1) hydrogen bonding between host and guest, (2) suitable cavity surrounded by polar moieties and (3) hydrophobic residue of guest dipeptides. Among these factors, the shape of the binding cavity may be readily modified by using mixed monolayers. We assumed that short acidic or basic groups are appropriate for forming new cavities upon mixing with oligopeptide polar groups. Our first choice was an equimolar mixture of amphiphiles $2C_{18}BGly_2NH_2$ and $2C_{18}BCOOH$.(ref. 6 and 7) Phase separation of the two components was not observed in their π–A isotherm. The π–A behavior also displays a positive deviation of 0.12 from that of the ideal mixture at a surface pressure of 25 mN/m where the monolayer was transferred onto solid substrates. The individual monolayers show intermolecular hydrogen bonding, as confirmed by FT-IR spectroscopy. In the mixed equimolar monolayer, IR results indicate that intermolecular hydrogen bonds of the individual monolayers are broken with new hydrogen bonds formed, due to mixing of the two components. Schematic representation of the receptor site is given in Fig. 4.

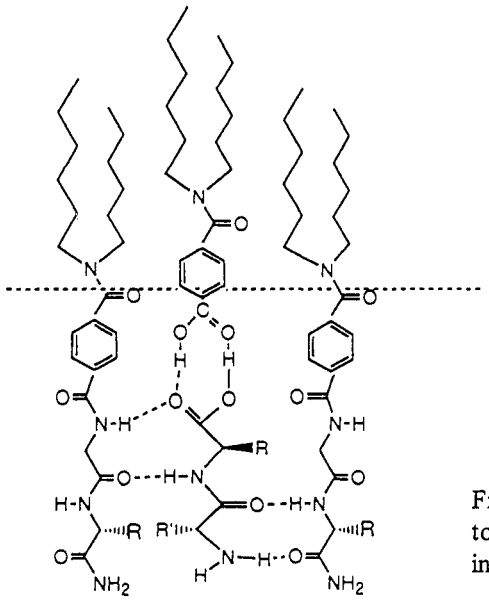


Fig. 4 Selective binding of aqueous dipeptides to a mixed peptide monolayer. C-terminal insertion.

The nature of the receptor sites

Key factors for dipeptide binding are cavity size, mode of hydrogen bonding, and disposition of guest hydrophobic group. As mentioned above, monolayers of single-chain derivatives of oligoglycines formed strong inter-peptide hydrogen bonding, and binding of guest peptides was not detectable. In the case of double-chain amphiphiles, dipeptide binding was not observed for monolayers of $2C_{18}BGlyYNH_2$ (Y = Ala, Val, Leu and Phe). Since molecular areas of these monolayers in the condensed phase are almost the same as that of $2C_{18}BGly_2NH_2$, monolayers with large amino acid side chains cannot provide cavities large enough for insertion of guest dipeptides. The nature of the binding cavity can be readily modified by using mixed monolayers. The binding to mixed monolayers of $2C_{18}BXYNH_2/2C_{18}BCOOH$ was detected for some dipeptides. The binding behavior depends on the size of side chains of amino acid residues in both of dipeptide guests and peptide monolayer hosts. Combination of large guest and large host,

bound to oxalate through hydrogen bonding and the other guanidinium existed as the non-hydrogen bonded counterion.
These results are useful for development of two-dimensional molecular patterns. In fact, AFM study indicates that dialkylguanidinium produces a monolayer in which alkyl chains are packed randomly.(ref.10) The chain packing becomes regular through their binding with polycarboxylates dissolved in the subphase.

Interaction of Melamine Monolayers with Barbituric Acid (ref. 11)

Monolayers containing the melamine function have been known to bind barbituric acid very efficiently. Since melamine monolayer $2C_{12}$Mela and barbituric acid have two hydrogen bonding faces, they can form a linear alternating complex. The XPS data of the transferred monolayer showed saturation binding of equimolar barbituric acid. Curve fitting according to the Langmuir adsorption isotherm revealed that the maximum binding ratio was 1.1 ± 0.1 with binding constant of 3000 M^{-1}. Barbituric acid in subphase (1 mM) caused an expansion of p-A isotherm with increase in the molecular area from 0.42 nm^2 to 0.45 nm^2 at the surface pressure of 15 $mN \cdot m^{-1}$. The AFM image of the methyl terminalwas observed almost all over the mica plate. The monolayer was not damaged by AFM tip even after repeated scanning. However, a hole could be artificially pierced through the monolayer with an applied force stronger than 10^{-9} N. The hole ,about 2 nm deep, is comparable to the calculated molecular length, and apparently represents the thickness of the monolayer. Clearly, brighter portions correspond to individual terminal methyl groups of the alkyl chain, and are regularly arranged in a two-dimensional oblique array with nearest-neighbor spacing of 0.40 ± 0.02 nm. The occupied molecular area of the monolayer component as evaluated from these data to be 0.19 ± 0.01 nm^2 per molecule is consistent with the supposition that one brighter portion in the AFM image is composed of one terminal methyl group.

Ordering of Monolayer Components Assisted by Multifunctional Template

We have demonstrated in previous studies that molecular monolayers can bind polar guest molecules dissolved in the aqueous subphase through complementary hydrogen bonding. These results were extended to double-site molecular recognition, in which a guest molecule was specifically bound at two functional sites to two monolayer components.(ref. 12) FAD molecule is composed of one isoalloxazine unit, two phosphate units and one adenine unit, which are complementary to triaminotriazine, guanidinium, and orotate, respectively. Figure 5 displays the mode of molecular recognition.(ref. 13). In the p-A isotherm, the limiting area for **TGO**(1:2:1) mixture is 1.33 $nm^2 \cdot molecule^{-1}$ in the presence of aqueous FAD, and is close to the minimal cross section of the total alkyl chains (1.1 - 1.3 $nm^2 \cdot molecule^{-1}$). The p-A curves of individual monolayers of **T** and **O** are not altered in the presence of 10^{-5} M FAD, probably due to low substrate binding. In fact, aqueous riboflavin and adenosine did not show efficient binding to monolayers of **T** and **O**, respectively, at this low substrate concentration. In contrast, aqueous FAD causes expansion of a monolayer of **G**. Combined ionic and hydrogen-bonding interactions between guanidinium monolayer and phosphate in FAD contribute to the strong binding.

for example LeuLeu guest and $2C_{18}BGlyValNH_2/2C_{18}BCOOH$ host, and that of small guest and small host, for example GlyGly guest and $2C_{18}BGly_2NH_2/2C_{18}BCOOH$ host, did not produce effective binding. In contrast, complementary combinations of large guest/small host, and those of small guest/large host, showed significant binding. These facts imply that size matching based on van der Waals contact between cavity and guest is essential for effective binding.

Induction of Recognition Site by Guest Binding

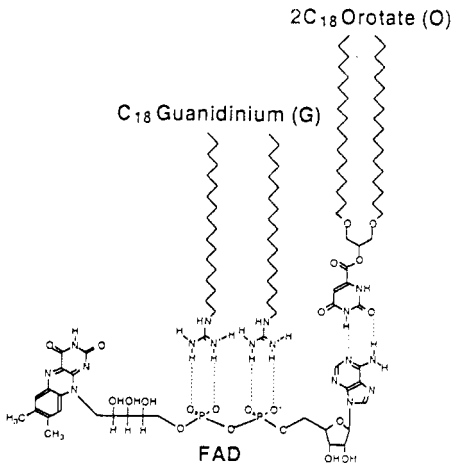
The experimental results for the mixed monolayer have an important implication for the formation of receptor sites. Two monolayer components, $2C_{18}BGly_2NH_2$ and $2C_{18}BCOOH$, are mixed well on pure water. The positive deviation in the molecular area and the IR spectral data indicate that. these two components are mixed randomly on pure water. In contrast, there is observed a specific 2:1 interaction (two host and one guest molecules) between host monolayer of $2C_{18}BGly_2NH_2/2C_{18}BCOOH$ and guest GlyLeu, as suggested by the Langmuir adsorption isotherm. Therefore, the guest binding must induce redistribution of monolayer components so as to produce a specific binding site. This is analogous to the "induced-fit" mechanism, in which binding of substrates to an enzyme active site causes conformational changes that align the catalytic groups in their correct orientation. This combinatorial recognition site is crudely analogous to the hypervariable region of antibodies. We believe that the existence of flexible recognition sites is characteristic of mixed monolayers.

TWO-DIMENSIONAL MOLECULAR PATTERNING

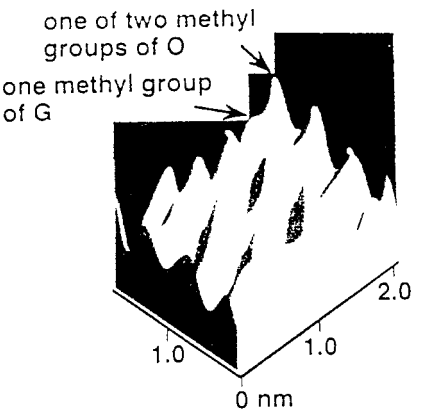
Designed formation of nanometer-scale molecular patterns should provide exciting possibilities in fundamental supramolecular chemistry and in molecular scale electronic devices. Several techniques that have been recently developed for two-dimensional patterning such as photolithography, laser manipulation and cantilever techniques do not supply a molecular resolution. Self assembly of molecules may be utilized for this purpose, if the assembly process is properly controlled. The traditional LB technique cannot be effective for the control of molecular arrangement within the two-dimensional plane. A new strategy must be added to the conventional LB technique, in order to achieve designed molecular arrangement with molecular precision.

Interaction of Guanidinium Monolayer with Carboxylate Guests(ref. 8)

Efficient and specific binding between host and guest is essential for disposition of component molecules within a mixed monolayer to be fixed at the air-water interface. Very strong binding constants (10^6—10^7 M^{-1}) were observed between a guanidinium monolayer and phosphate derivatives such as AMP or ATP.(ref. 9) The interaction between guanidinium and carboxylate should be similarly effective. We examined the interaction between monolayers of octadecyl- and dioctadecylguanidinium amphiphiles and polycarboxylates dissolved in subphase on the basis of p(π-A isotherm, FT-IR spectroscopy, and XPS measurements. Expansion of the molecular area was greater for the octadecylguanidinium monolayers than for the dioctadecylguanidinium monolayers. When linear dicarboxylates were bound to the interface, molecular areas of the monolayer increased, as the length of the methylene chain between carboxylate groups increased. The molecular packing was affected by the shape of polycarboxylate molecules in the case of phthalate, *cis*-1,2-cyclohexanedicarboxylate, and 1,1-cyclohexanediacetate. Apparently, the molecular packing in the complexed monolayers is governed by the distance and relative orientation of the two carboxylate groups in the guest molecule. With all the dicarboxylates excluding oxalate, the formation of 1:1 guanidinium/carboxylate pairs with hydrogen bonding interactions is indicated. Oxalate produced an asymmetric complex where one guanidinium was



(a)



(b)

Fig. 5(a) A scheme of multi-site molecular recognition between FAD and a **G/O** mixed monolayer at the air-water interface. (b) Three-dimensional AFM image (1.8×2.1 nm^2).

The elemental composition of an LB film of **TGO**(1:2:1) monolayer on aqueous FAD agrees most closely with the calculated value for the composition of **TGO**·FAD(1:2:1:1). The absence of sulfur and sodium indicates that ion exchange of the counterion of **G** (*p*-toluenesulfonate) with FAD is complete. FAD binding was almost stoichiometric even when the FAD concentration was lowered to 10^{-6} M. We can conclude from these results that there exists multiple molecular recognition such as depicted in Figure 5.

We can assume from these results that it is possible to produce molecularly defined patterns from monolayer components by using multi-site guest molecules. Figure 6 shows a three-dimensional AFM image of the **G/O** mixed monolayer on aqueous FAD with a scan area of 1.8 2.1 nm^2.(ref. 14) The AFM image revealed that the higher portions of the **G/O** mixed monolayer on aqueous FAD are regularly aligned with a distorted hexagonal array. The AFM image also displays a periodic wave-like structure composed of two different peaks. The height difference between the two peaks is several angstrom. The binding of FAD with the mixed monolayer as illustrated in Figure 5 would cause a height difference between the two terminal CH_3 groups. Hence, the higher and lower peaks in the AFM image may be assigned to the **O** and **G** molecules, respectively. Thus, a regular arrangement of the methyl terminals of **O** molecule is seen as a molecular pattern in the mixed monolayer. On the other hand, the AFM image on pure water revealed a periodic wave-like pattern composed of only one kind of peak corresponding to individual methyl terminals of **G** or **O** molecule. They are regularly arranged in a hexagonal array, indicating that the height of the terminal CH_3 group of the **G** and **O** component is the same. The latter results are derived from regular spatial alignment of the same C_{18} chains of the two components above the water surface. The longer polar region of the O

component would then be buried in the aqueous subphase deeper than the shorter guanidinium unit. It is clear that the alkyl chain packing is altered by FAD binding.

CONCLUSION

We have demonstrated in this article that complimentary hydrogen bonding at the air-water interface leads to unique supramolecular systems. It is surprising that highly specific receptor sites are formed by spontaneous assembly of single and multiple monolayer components. This finding provides an exciting possibility of *de-novo* design of artificial protein surfaces at the interface. The starting functional units would not be limited to peptides, since the effectiveness of the interfacial hydrogen bonding is quite general. The formation of molecular patterns should also give rise to versatile possibilities. Although molecularly defined surfaces are abundant in the biological systems, there are no good precedents in the artificial molecular surface. Further elaboration of template structures are required.

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