Multi-Site Binding of Aqueous Dipeptides by Mixed Monolayers at the Air-Water Interface

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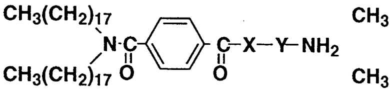
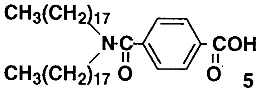
Aqueous dipeptides are bound to two-component monolayers which contain oligopeptide polar units together with benzoic acid or pyridine head groups. Binding of guest dipeptides to the mixed monolayers depends specifically on the nature of interfacial cavities which can be controlled by combination of polar head groups. The binding ratio is lessened with increasing sizes of the side chain of the Y residue in the Gly-Y dipeptide moiety of the host amphiphiles.

Molecular recognition of peptides by synthetic receptors is one of the most actively studied fields both in biochemistry and in organic chemistry,[1-6] due to its implications in the biological process and its potential biomedical applications. Recently, we have shown that complementary hydrogen bonding acts as a means of molecular recognition of aqueous dipeptides by functional monolayers in spite of the presence of bulk water.[7] In this case, several GlyX dipeptides were efficiently bound to monolayers of oligoglycine amphiphiles, whereas dipeptides of XGly or XX' type (X, X' = neutral amino acid residues other than Gly) were not bound probably because of steric crowding (for XX' dipeptides) or weak hydrogen bonding (for XGly dipeptides) at the binding site. In the present work, we describe that new binding sites towards dipeptides are created by mixed monolayers of oligopeptide amphiphiles and benzoic acid or pyridine amphiphiles.[8] The nature of binding cavities is influenced by the side chain of amino acid residues of oligopeptide amphiphiles.



X, Y = amino acid residue
1; (X, Y) = (Gly, Gly)
2; (X, Y) = (Gly, Val)
3; (X, Y) = (Gly, Leu)
4; (X, Y) = (Gly, Phe)



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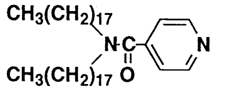

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Efficient binding of aqueous GlyX dipeptides by the single-component oligoglycine monolayer was promoted by three key factors: (1) hydrogen bonding between host and guest, (2) suitable cavity surrounded by polar moieties and (3) hydrophobicity of guest dipeptides.[7] Among these factors, the nature 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 **1** and **5**. 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 (dimeric COOH for monolayer **5** with $\nu_{C=O}$ 1701 cm^{-1}, and hydrogen bonded oligoglycine units for monolayer **1** with ν_{NH} 3309 cm^{-1}). In the mixed equimolar monolayer, the ν_{NH} peak is shifted to 3320 cm^{-1}, and the COOH peak becomes a broadened shoulder at 1700 to 1720 cm^{-1}. These IR results indicate that intermolecular hydrogen bonds of the individual monolayers are broken down, due to mixing of the two components.

Table 1. Binding ratio of dipeptides towards monolayers as determined by XPS[a]

Monolayer	Guest/Host (mol/mol)[b]				
	GlyLeu	LeuGly	LeuLeu	HisLeu	GlyGly
1	0.33	0.00	0.00	0.00	—
5	0.17	0.17	0.00	—	—
6	—	—	—	0.00	—
1/5(1:1)	0.41	0.45	0.45	—	0.00
1/6(1:1)	—	—	—	0.13	—
2/5(1:1)	0.34	—	—	—	0.39
3/5(1:1)	0.12	—	—	—	—
4/5(1:1)	0.00	—	—	—	—

[a] LB films of 14 layers were used. [b] The concentration of aqueous guests was 10 mM except for HisLeu (5 mM).

Guest dipeptides dissolved in subphase such as GlyLeu caused expansion of the π–A curve of mixed monolayer **1/5** (figure not shown), suggesting the interaction of GlyLeu with the monolayer. Monolayer **1/5** was subsequently transferred onto gold-deposited glass slides in the vertical mode. The transfer ratio of the monolayer was 1.0 ± 0.1 in the up-stroke mode and 0.4 ± 0.1 in the down-stroke mode from pure water. It was 1.0 + 0.1 in the up-stroke mode but there was no transfer in the down-stroke mode from 0.01 M dipeptide subphases. Elemental composition was determined from the C/N ratio (± 10%) in X-ray photoelectron spectroscopy (Perkin-Elmer PHI 5300, X-ray source MgKα 300 W, take off angle 45°).

Table 1 summarizes the binding behavior. Monolayer **1** can bind GlyLeu efficiently, but not LeuGly and LeuLeu, as reported previously,[7] and monolayer **5** can adsorb small amounts of GlyLeu and LeuGly indiscriminately. In contrast, the equimolar mixture of **1/5** can bind these three dipeptides efficiently. Saturation binding behavior observed for GlyLeu guest is depicted in Figure 1, together with a plausible model of host/guest interaction. Curve fitting to the Langmuir isotherm gives rise to a binding constant of 475 M^{-1} and a saturation guest/host ratio of 0.47.

FTIR spectra of the LB films show that a broadened shoulder at 1700—1720 cm^{-1} (see above) is lessened with increasing incorporation of GlyLeu guest, implying the benzoic acid group is hydrogen bonded with the guest. A peak corresponding to the COO$^{-}\cdots$NH$_3^{+}$ salt bridge was not observed around 1610—1570 cm^{-1}. Characteristic IR bands for bound GlyLeu include an unionized but hydrogen bonded COOH peak at 1690 cm^{-1} and a shift of the ν_{NH} band to 3309 cm^{-1} due to hydrogen

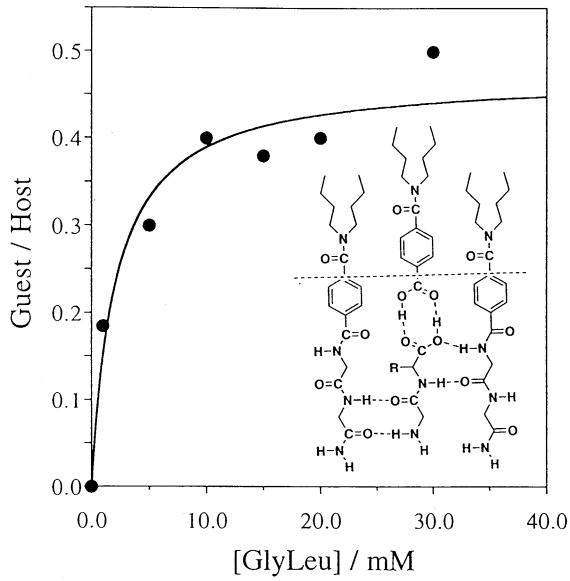

Figure 1. Binding curve of GlyLeu to an equimolar monolayer of **1**/**5**, and a schematic illustration of the host/guest interaction.

bonding with host. These IR results are satisfied by the interaction model included in Figure 1.

Monolayer component **5** can bind HisLeu by acid–base interaction of the His side chain, but it does not operate between HisLeu and component **6**. Therefore, it is notable that HisLeu is bound by a mixed monolayer **1**/**6** (see Table 1). The pyridine unit in **6** would form hydrogen bonding with the proton donor moiety of the guest, and the nature of the binding site of monolayer **1**/**6** must be rather different from that of **1**/**5**.

The binding of GlyLeu depends on the combination of monolayer components. As shown in Table 1, its binding is lessened with increasing sizes of the side chain of the Y residue in the Gly-Y dipeptide moiety. In fact, no GlyLeu binding was observed when Gly was replaced by Phe in the mixed monolayer. In contrast, highly hydrophilic, slim GlyGly guest is not bound to the **1**/**5** monolayer under the conditions used. However, this dipeptide becomes stably bound to the mixed monolayer if the dipeptide moiety in the monolayer is changed from GlyGly to GlyVal.

All these binding data are consistent with our view that specific sites are created at the interface due to intermolecular hydrogen bonding of oligopeptide chains. The peptide binding is stoichiometric and depends specifically on the nature of the self-assembled receptor site.

References and Notes

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8 Syntheses of **1** and **5** were described in Ref 7. Syntheses of the other lipids will appear in a future publication. Compound **2**: colorless powder; mp 206.0—207.0 °C. Anal. Found: C, 73.42; H, 11.20; N, 6.61%. Calcd for $C_{51}H_{92}N_4O_4\cdot 1/2H_2O$: C, 73.42; H, 11.24; N, 6.72%. Compound **3**: colorless powder; mp 42.5—43.5 °C. Anal. Found: C, 73.69; H, 11.21; N, 6.55%. Calcd for $C_{52}H_{94}N_4O_4\cdot 1/2H_2O$: C, 73.62; H, 11.29; N, 6.60%. Compound **4**: colorless powder; mp 91.8—92.3 °C. Anal. Found: C, 74.96; H, 10.66; N, 6.08%. Calcd for $C_{55}H_{92}N_4O_4\cdot 1/2H_2O$: C, 74.87; H, 10.62; N, 6.35%. Compound **6**: colorless powder; mp 60.5—61.0 °C. Anal. Found: C, 80.19; H, 12.39; N, 4.38%. Calcd for $C_{42}H_{78}N_2O$: C, 80.44; H, 12.54; N, 4.47%.