Ordering of Dipeptide Molecules on Guanidinium Bilayer Membrane

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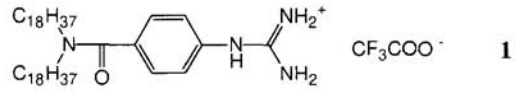
(Received August 3, 1998; CL-980592)

Dipeptide molecules were shown by induced circular dichroism to bind in specific manners on guanidinium-functionalized bilayer membranes in 40 vol% ethanol/water. The carboxylate-guanidinium interaction determined the peptide alignment.

Binding of peptide molecules on membrane surfaces attracts much attention, because of its implications as models of biological receptors and as a technological basis of biosensors. Most of sequence-selective peptide receptors that have been artificially synthesized in the past do not incorporate interfacial features characteristic of many of the biological recognition.[1] We have studied specific binding of water-soluble, small peptides to peptide monolayers. The efficiency of binding was strongly affected by the number of alkyl chains attached to a peptide functional unit : monolayers of single-alkyl peptide amphiphiles were incapable of binding of guest peptides,[2] whereas monolayers of double-alkyl peptide amphiphiles showed selective binding of aqueous dipeptides.[3] Furthermore, mixed monolayers of peptide amphiphiles and second functional amphiphiles (carboxylate and guanidinium)[4,5] displayed enhanced peptide binding due to cooperative action of the peptide and second functional units. In particular, the mixed monolayer with guanidinium function led to much enhanced binding thanks to strong interaction of guanidinium and peptide C-terminal.

The latter result gives an interesting implication that guanidinium alone is sufficient to align peptide molecules on the surface of molecular assembly. We, therefore, examined in this study the interaction of dipeptides with aqueous bilayer assemblies of guanidinium amphiphiles.



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We chose the same guanidinium amphiphile,**1**, that was used as monolayer in the previous paper.[5] It can be dispersed in water and in aqueous ethanol to give bilayer membrane. Electron microscopic observation (Hitachi H600) indicated the presence of mutilamellar sheets in both media. Differential scanning calorimetry (Seiko SSC/5200H) gave phase transition peaks of bilayers at 43 °C in water and at 48 °C in 40 vol% ethanol/water.

Then, we looked for conditions where the bilayer would most effectively bind guest peptides. As shown in Figure 1, the guanidinium bilayer(30 μM) gives induced circular dichroism in the presence of Gly-L-Ala(1 mM) with the negative Cotton effect at 256 nm. Gly-D-Ala gave a mirror image CD spectrum. Interestingly, the ICD was not observed in a pure water medium. It was enhanced at 30 - 40 vol% ethanol/water mixtures, and was greatest at 40 vol%. We have shown in other bilayer systems that chiral interaction of bilayers and chromophoric guests is most pronounced at this ratio of ethanol and water.[6] Therefore, this

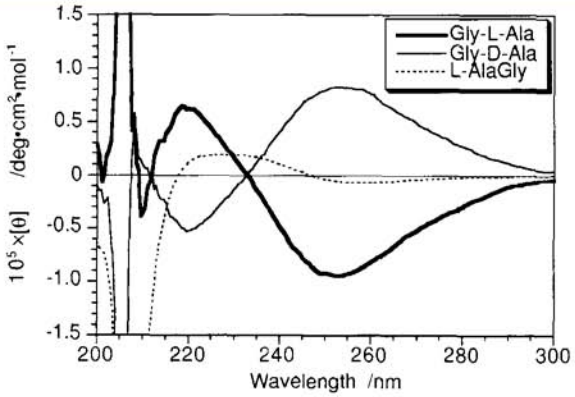


Figure 1. CD spectra of bilayer (**1**) with Gly-L-Ala or Gly-D-Ala in ethanol/water (2:3 v/v) ; 20 °C, [**1**] = 30 μM, [dipeptide] = 1 mM. CD Spectra of dipeptides alone are subtracted. Instrument, Jasco J-720.

mixture was selected for the subsequent experiments. Dipeptides are dissolved in the zwitter ionic form under these conditions. CD of dipeptides themselves are negligibly small in the UV absorption region of **1** (240 - 260 nm).

The CD intensity increases with the dipeptide concentration, and is saturated at 1 mM with molar ellipticity([θ]) of 6×10^4 deg•cm^2•mol^{-1}: Figure 2a.The [θ] values were used to determine the binding constant of Gly-L-Ala based on the Benesi-Hildebrand analysis ; however, a linear relation was not obtainable. Thus, we used the Hill plots that have been used to analyze the cooperative binding of O_2 toward hemoglobin. The cooperative binding of peptide(P) toward bilayer(B) is expressed by

$$B + nP \overset{K}{\rightleftharpoons} B(P)_n \qquad (1)$$

and the following relation holds

$$Y / (1 - Y) = K [P]^n \qquad (2)$$

where the degree of binding saturation, Y, is given by $Y = [B(P)_n] / [B]_0$.

The logarithmic plots of Eq. (2) gives a linear relation, as shown in Figure 2b, and n = 1.7 and $K = 2500$ M$^{-1.7}$ are obtained from slope and intercept, respectively. This result is an indication of positive cooperativity in the peptide binding process, showing that a Gly-L-Ala molecule bound to the guanidinium surface promotes subsequent peptide binding. The promotion may be caused by dipolar and hydrogen-bonding interactions with the neighboring peptides. A similar cooperativity has been found for the complementary binding of adenine to an orotate monolayer due to stacking of bound adenine.[7]

Figure 3a compares molar ellipticities (at 256 nm) of the bilayer in the presence of selected peptides at 1 mM, where binding of peptides are essentially saturated. It is noted that GlyGly-L-Ala and L-alanine do not give ICD spectra. The absence of the CD

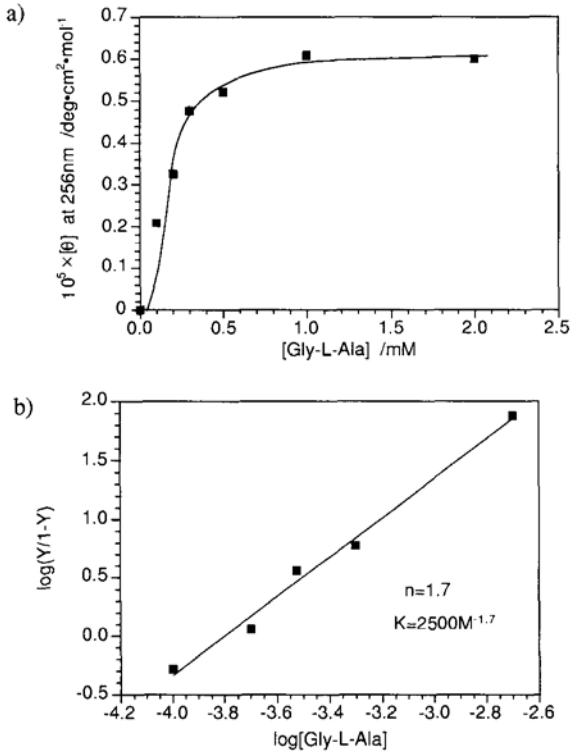
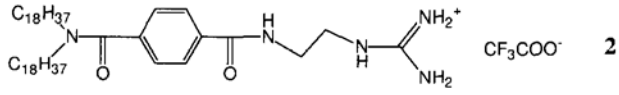


Figure 2. a) Binding curve of Gly-L-Ala to guanidinium bilayer **1** in ethanol/water (2:3 v/v) ; 20°C, [**1**] = 10 μM. b) Hill plots.

signal for L-alanine may be explained by its weak binding and/or by bound alanine molecules not being aligned enough to give detectable ICD. It is curious that this tripeptide does not give ICD. It may as well be bound to the guanidinium bilayer stronger than dipeptides. One possibility is that stronger hydrogen bonding among the Gly residues in the guest makes juxtaposition of the Ala residue relative to the benzene chromophore less favorable. Among the dipeptides used, the ICD value is suppressed with increasing sizes of the amino acid residue in the series of Gly-L-X. Large side chains in X appear to interfere with juxtaposition of the chiral center relative to the benzene chromophore. Some of the dipeptides gave reversed ICD patterns, indicating that the spatial disposition of their chiral carbons is reversed relative to those of other dipeptides. These dipeptides (Gly-L-Asp, Gly-L-Glu) possess side-chain functional groups and their interaction with the guanidinium in place of C-terminal carboxylate must produce altered spatial arrangements. In fact, Gly-L-Asn and Gly-L-Gln which do not have free carboxyl groups in the side chain gave Cotton effects similar to non-functionalized side chains. These experimental results may be explained by the schematic arrangement of guest peptides shown as Figure 3b.

The short distance between the chiral carbon and the benzene chromophore is crucial for the observation of ICD, since the use of bilayer **2**[8] instead of bilayer **1** does not give measurable ICD, and L-AlaGly whose chiral carbon is far removed from the benzene chromophore upon binding gives a less well defined CD spectrum (Figure 1).



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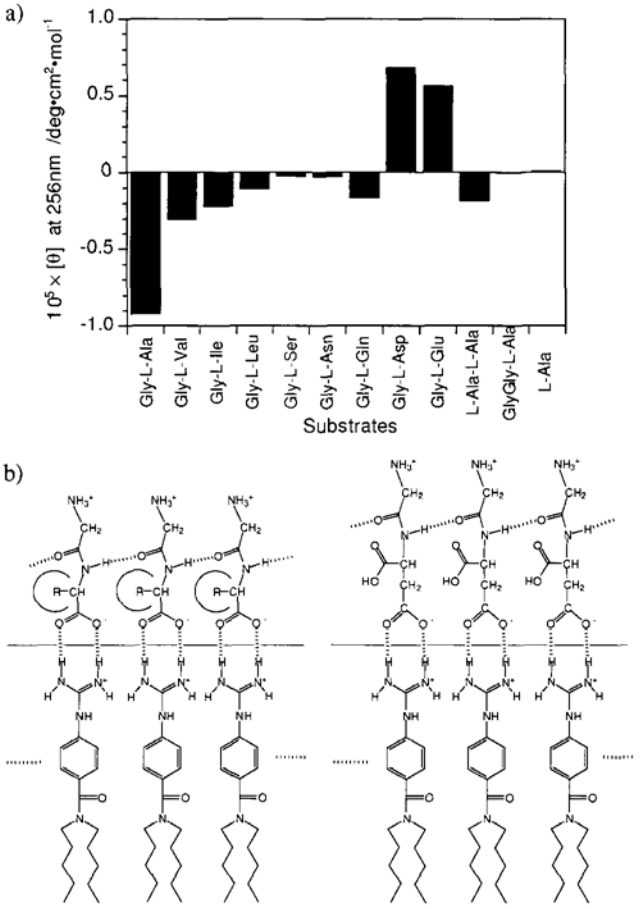

Figure 3. a) Molar ellipticity ([θ]) at 256 nm for bilayer (**1**) with various substrates in ethanol/water (2:3 v/v) ; 20 °C, [**1**] = 30 μM, [substrate] = 1 mM. b) Schematic representations of binding of aqueous dipeptides to guanidinium bilyer (**1**) ; left : non-acidic dipeptides (Gly-L-X), right : acidic dipeptides (Gly-L-Asp).

In conclusion, dipeptide molecules are organized on the surface of guanidinium bilayers according to their specific structures. Ordering of peptide moieties that are bound covalently to bilayer membranes has been examined in the past.[9,10] It is also possible to order peptide molecules without covalent bonding.

Reference and Notes

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