Molecular Recognition of Biotinyl Hydrophobic Helical Peptides with Streptavidin at the Air/Water Interface†

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We report here on the issue of molecular recognition by a peptide monolayer which is formed at the air/water interface.

Peptides are regarded as the ideal unit for construction of biologically functional molecular systems, because they take a definite conformation[1] and can be functionalized by chemical modifications at side chains[2] or chain terminals.[3] It has previously been reported that a hydrophobic peptide, Boc-(Ala-Aib)$_8$-OMe, took an α-helical conformation as evidenced by X-ray diffraction analysis of the crystalline structure[4] and that the analogues modified at the chain terminus formed a stable monolayer at the air/water interface.[5] The monolayer technique is the basis of the Langmuir–Blodgett method, which might be useful for the preparation of functional thin layers.

The biotin and streptavidin (SAv) system is a well-known biological receptor/ligand pair,[6] having a specific and strong interaction ($K_d = 10^{-15}$ mol/L).[7] Many lipid derivatives having a biotin group were spread at the air/water interface, and upon addition of SAv to the subphase, a 2-dimensional (2D) crystal of SAv was formed at the interface, due to binding of SAv to the lipid.[8-10]

Two kinds of peptides having a biotin group, **1** and **2** (Figure 1), were synthesized by the reaction of the amino end group of the helical peptide[5] with biotin aminocapronate *N*-hydroxysuccinimide (Sigma, St. Louis, MO). A hydrophilic peptide spacer, trisarcosine, is inserted between the amino terminus of the helical peptide and the biotin group in **1**; no spacer is used in **2**. The CD spectra of both peptides[11] showed the typical double-minimum pattern in ethanol/water (95/5 v/v), indicating that both peptides take an α-helical conformation.[12]

The inactive SAv was prepared by treatment of SAv with 5 mol equiv of biotin in water to block its binding sites, followed by gel filtration through a G-25 column. Streptavidin was labeled with the isothiocyanate of fluorescein (FITC-SAv)[13] or rhodamine (Rh-SAv).[14]

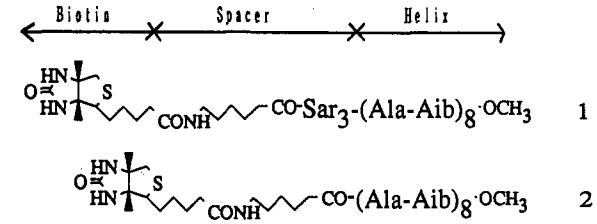

Figure 1. Molecular structure of the biotinyl peptides **1** and **2**.

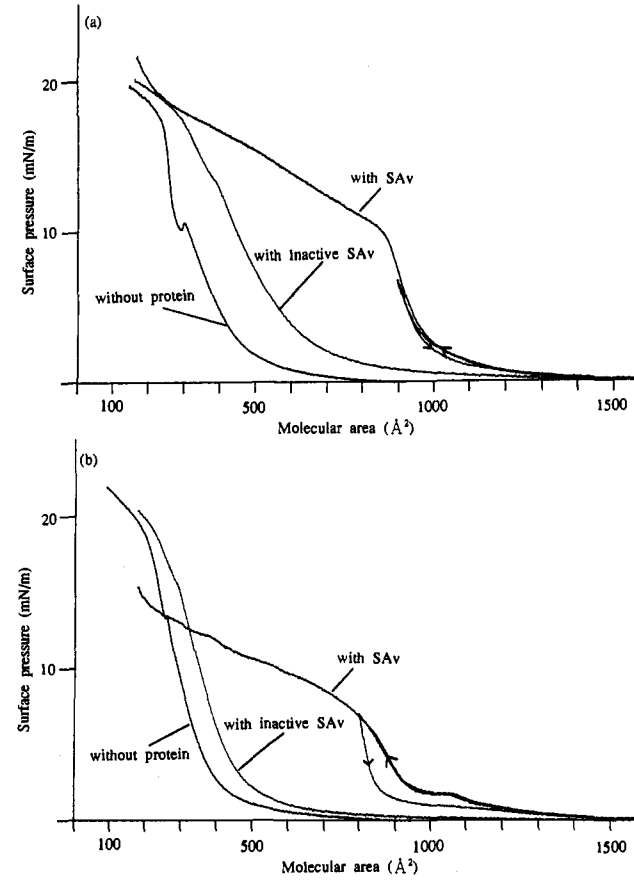

Figure 2. Surface pressure–area isotherms of (a) **1** and (b) **2** interacting with SAv or inactive SAv or in the absence of protein. The curves of compression, expansion, and recompression in this order are shown together with arrows in the presence of active SAv.

The surface pressure–area (π–A) isotherm was measured by using a homemade Langmuir trough with 490 cm^2 surface area[15] at a compression rate of 0.25 cm^2/s. The isotherms of both peptides show an inflection point at 250 Å^2 in the absence of the protein in the subphase, which should be collapsion point of the monolayer[5] (Figure 2a and b, without protein). The molecular area of the inflection was in agreement with the cross section along the helix axis of the helical part, which was determined by X-ray analysis.[4] The observation strongly suggests that the helical parts of both **1** and **2** are oriented with the helix axis parallel to the interface.[5] It is notable that a small mound was observed around 300 Å^2 in the isotherms of both peptides under the compression process, which is ascribed to the property of the

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peptides to associate in an orderly form.[5,16] This is considered to explain the mound observation in the isotherm, in contrast to the biotinylated lipids.

SAv or inactive SAv was injected into the subphase, and the mixture was incubated for 1 h at 30 °C, followed by cooling to 20 °C. π–A isotherms of **1** (Figure 2a) and **2** (Figure 2b) with active or inactive SAv were measured. Nonspecific interaction of SAv with the interfacial film can be estimated from the effect of the inactive SAv on the isotherm.[8] The addition of inactive SAv caused a shift of the isotherm curve to the larger area, indicating nonspecific adsorption to the monolayer. It seems that more of the inactive SAv was adsorbed to the biotinyl peptide monolayer than to the biotinyl lipid monolayer,[8] probably because the biotinyl peptide is more similar to SAv than the biotinylated lipid in chemical properties. The N-terminus of the helix part in **1** should be pulled into the water subphase due to the hydrophilic spacer, trisarcosine, more strongly than that in **2**. This should explain why **1** showed a slightly larger shift than **2** in the π–A isotherm upon interaction with inactive SAv, which reflects the degree of nonspecific interaction. **1** exposes a larger molecular surface to the water subphase, which promotes access of streptavidin in the subphase to the peptide. The specific interaction was estimated by the difference between the isotherms in the presence of active SAv and inactive SAv. Obviously, the presence of active SAv increased the molecular area more strikingly than the presence of inactive SAv, indicating the specific binding of SAv to both peptides, **1** and **2**, at the interface. The π–A isotherms were measured upon compression, expansion, and recompression in the presence of active SAv, but no hysteresis was detected in the complex formation (Figure 2). The agreement of the recompression curve with the first compression one in Figure 2a and b means that the protein–peptide complex monolayer is stable. The amount of SAv bound to the monolayer of **1** was nearly the same as that bound to the monolayer of **2**. The biotin moiety of **1** should stay at the aqueous phase due to the presence of the hydrophilic spacer, resulting in an easy access to SAv in the aqueous phase.

The peptide (**1** or **2**) in $CHCl_3/CH_3OH$ (9:1 v/v) at varying concentrations was spread at the air/water interface of a small trough (10 cm^2) in the range of 1500–500 $Å^2$/molecule (a gas–analog state). FITC-SAv was injected into the aqueous subphase (0.5 M NaCl), followed by incubation for 1–18 h. In the case of **1**, domains of the FITC-SAv were observed (Figure 3) by fluorescence microscopy,[17] which displayed distinct fluorescence anisotropy on irradiation of polarized excitation light. The anisotropy is caused by the microscopic orientation of the fluorescence probe attached to the SAv molecule and strongly suggests that the SAv molecules are highly ordered and form the 2D crystal domain of SAv.[8] When the Rh-SAv was used, a similar anisotropic domain formation was observed. However, in the case of **2**, the domain formation was not observed, and only the homogeneous fluorescence was done with either FITC-SAv or Rh-SAv. The π–A isotherm of **2** was similar to that of **1**, reflecting similar binding behaviors with SAv (Figure 2). Therefore, no 2D crystal of SAv was formed from **2**, because the short spacer chain in **2** did not allow the crystalline arrangement of SAv. This result is different from the case with the biotinylated lipid monolayer performed before.[8] The biotinylated lipid without a proper spacer did not induce formation of a 2D crystal of SAv because SAv could not bind to the biotin group of the lipid spread at the air/water interface.[8] In the case of the peptide monolayer, however, the peptide **2** did not influence formation of a 2D crystal of SAv, although SAv binds to the peptide similarly as to the peptide **1**, as evidenced by the isotherms shown in Figure 2. The different result on 2D crystal formation between the biotinylated lipid and peptides is ascribed mostly to the different molecular

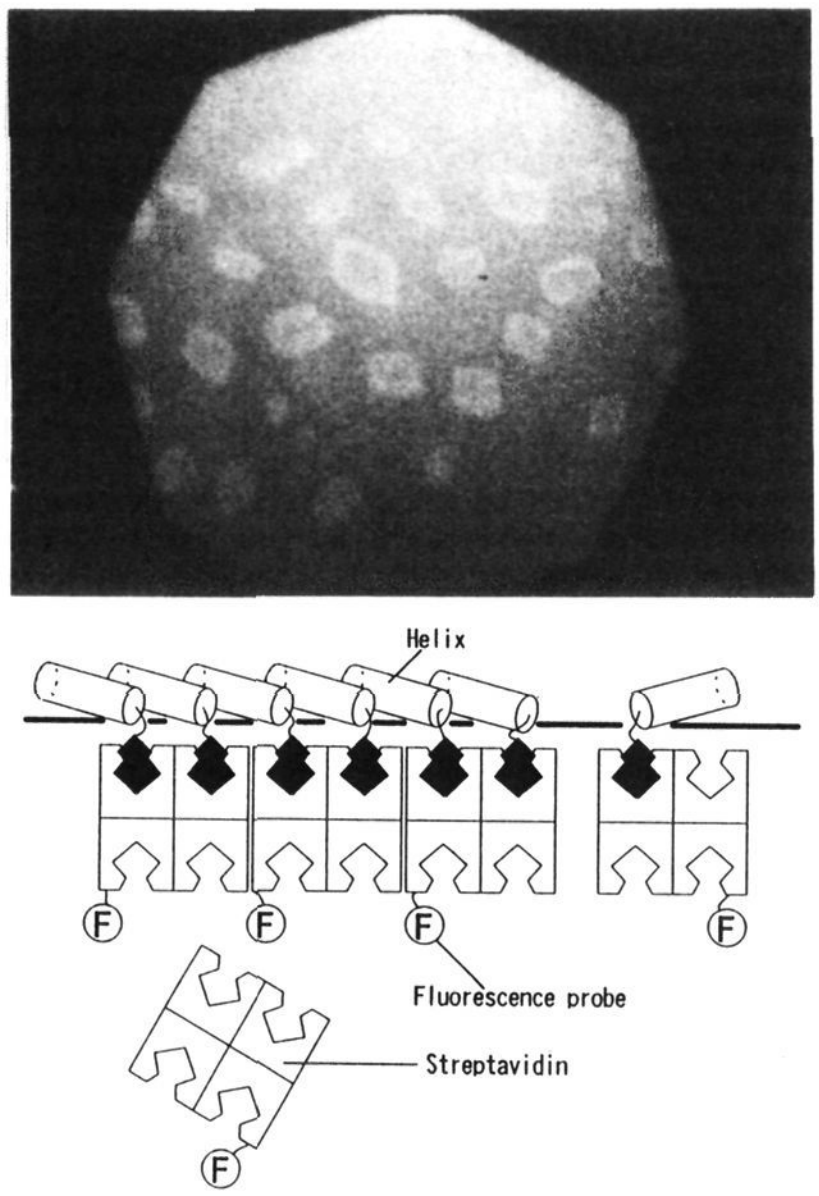

Figure 3. Fluorescence micrograph and schematic representation of the complex of **1** and FITC-SAv at the air/water interface.

area occupied at the air/water interface. The molecular area of SAv facing the interface, which contains two binding sites for biotin at the distance of 20 Å, is estimated to be 55 × 45 $Å^2$.[18] The helical peptide was spread at the air/water interface with a parallel orientation of the helix axis to the interface.[5] The length of the helix is 25.7 Å,[4] which is longer than half of the shorter side of the SAv cross section facing to the interface. Therefore, the peptide bound to SAv might cause steric hindrance, preventing another peptide from binding to a nearby SAv. This should be the case for **2** and should inhibit the crystallization of SAv. On the other hand, the molecule of **1**, with a longer, flexible spacer chain, may reserve the molecular flexibility even after binding to SAv, which enables it to accommodate the aligned helices which are bound by SAvs accompanying the crystallization of SAv.

The hydrophobic helical peptide with a biotin group behaved as the molecular recognition unit at the air/water interface. The spacer between the biotin group and the amino end of the peptide chain is necessary for the biotinyl peptide monolayer to function like lipid-based amphiphiles. The difference between **1** and **2** in the formation of the protein 2D crystal should be ascribed to the spacer length. However, we cannot exclude other effects of the spacer insertion into the biotin–peptide on the 2D crystal formation. For example, the molecular polarity, the affinity for the water phase, and the packing properties of the peptide might be changed by the hydrophilic spacer and therefore might result in the different packing state of SAv.

Supplementary Material Available: CD spectra of **1** and **2** (1 page). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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