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Multipoint Recognition of Basic Proteins at a Membrane Model

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In nature, cell surfaces receive numerous signals from the extracellular milieu and transduce them into their cytosol. These recognition events are mediated by specific receptors embedded in the cell membrane, which are highly sensitive to messengers such as nutrition components, hormones, proteins, or whole viruses.[1] Usually single weak interactions are multiplied and thereby drastically reinforce the simultaneous interaction with several binding sites on the cell surface.[2] This polyvalency simultaneously leads to increased specificity, allowing for a gradual or stepwise response to an external signal. It is state-of-the-art to reconstitute natural receptors in lipid bilayers[3] or to bind tagged proteins to lipid monolayers.[4] Very few reports, however, have appeared about artificial receptors in membrane models,[5] and virtually nothing is known about a biomimetic version of the polyvalent self-assembly of those systems.[6]

We recently discovered that calixarene tetraphosphonate **1** has a high affinity in methanol for *N*/*C*-protected arginine ($\sim 10^4$ M^{-1}) and lysine ($\sim 10^3$ M^{-1}) derivatives (Figure 1a).[7] No other amino acid side chain produced complexation-induced shifts in NMR titrations ($\Delta\delta_{max} < 0.02$ ppm).[8]

Since the tetraanionic receptor molecule has an amphiphilic structure with polar headgroups at the upper rim and nonpolar butoxy tails at the lower rim, we investigated its compatibility with the chemical environment of a lipid monolayer. Addition of increasing concentrations of tetraphosphonate **1** to a stearic acid monolayer on water led to incorporation of increasing amounts of receptor molecule in the monolayer (Figure 1c). The host molecules are evenly distributed among the surrounding excess lipid molecules, indicated by a smooth picture in a Brewster angle microscope.[9] Subsequent injection of arginine and lysine derivatives into the aqueous subphase produced moderate but distinct additional expansions of the pressure/area diagrams. Negative controls proved that in all cases, the stearic acid monolayer showed no interaction with any of the ligands. The absence of a compression plateau around 28 mN/m in the π-A diagram strongly indicates the obvious parallel orientation of the calixarene amphiphiles inside the monolayer.[10]

Since multipoint binding represents the key to specific and efficient biological recognition on cell surfaces, we attempted to imitate this process by offering polytopic analytes in the subphase which require an automatic receptor self-assembly (Figure 1b).[11] For a simple test, we used free mono-, di-, and triarginine and observed remarkable increasing expansions in the pressure/area diagram, confirming beautifully the hypothesis outlined above. Even at 10^{-7} M concentrations, triarginine could be clearly detected in water with only 0.13 equiv of embedded receptor molecule (Figure 2). Kunitake et al. recently described elegant mixed monolayers with sequence selectivity for dipeptides, albeit at millimolar concentrations.[12]

We explain the observed large shifts by reincorporation of additional host molecules with their guests from the subphase into the monolayer, after their charges have been mutually neutralized.

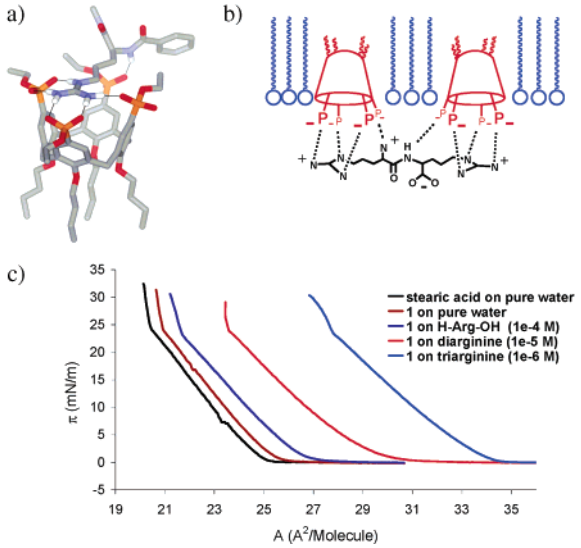

Figure 1. (a) Calculated complex structure between *N*/*C*-protected arginine and calixarene tetraphosphonate **1** (Monte Carlo simulation in water, MacroModel 7.0, 3000 steps). (b) Schematic representation of the multipoint binding mode in the case of diarginine recognition by the monolayer. (c) Pressure/area isotherms for arginine (10^{-4} M), diarginine (10^{-5} M), and triarginine (10^{-6} M) on the Langmuir film balance. Receptor **1** (0.13 equiv) was embedded in the stearic acid monolayer. Note the drastic expansions of the monolayer despite the decreasing peptide concentrations in the subphase.

This promising result prompted us to move on to larger peptides and proteins. In principle, such proteins with a high content of arginines or, to a lesser extent, lysines on their surfaces should be able to exert multipoint binding with the "mobile" immobilized arginine binder (Figure 2a). Indeed, the lysine-rich fraction of histone H1 as well as the arginine-rich cytochrome C displayed pronounced shifts in their respective pressure/area diagrams, even at 10^{-9} M concentrations in the subphase (Table 1). Only highly efficient protein/protein interactions are still effective at these low concentrations in the nanomolar range.[13] LB techniques drew 160 superimposed monolayers from the air/water interface. Direct UV spectroscopic measurements reveal the presence of cytochrome C's porphyrin band at 400 nm proportional to the amount of embedded **1**, and thus they provide evidence for the tight and specific protein binding (Figure 2b). The observed increase in surface area is roughly independent of the salt concentration, which was varied between 0.5 and 150 mM. Likewise, the variation of HEPES buffer concentration from 0.5 to 50 mM has a negligible effect on the pressure area diagrams.[14] Proteins with neutral or acidic surfaces should bind much more weakly to the calixarene receptor in the monolayer. Indeed, the Dps dodecamer[15] and an acyl carrier protein displayed only small shifts in the pressure/area diagram. For a better comparison, we examined all proteins at $\sim 10^{-8}$ M concentration and plotted the resulting isotherms in one diagram (Figure 2c).[16] Under these circumstances, most proteins interact only weakly with

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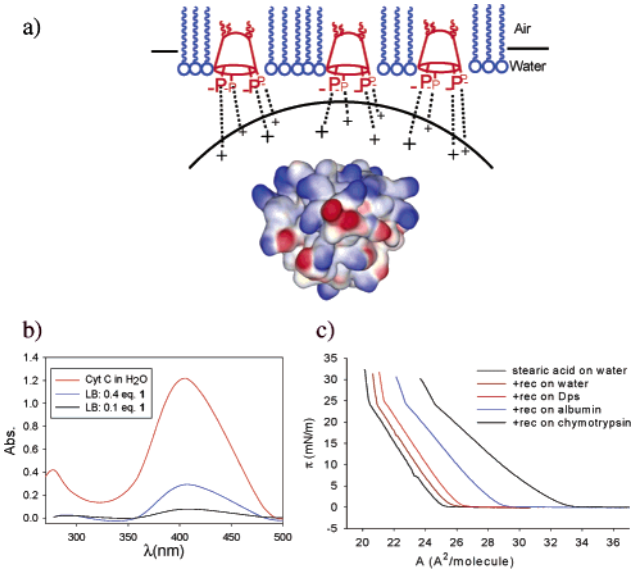

Figure 2. (a) Schematic representation for the proposed multipoint binding process: the receptor molecules self-organize in the fluid monolayer over the positive domains on the protein surface; the Connolly surface of cytochrome C is patterned with the ESP, showing basic (blue) and acidic domains (red) on the protein surfaces. (b) UV/vis spectra of cytochrome C in water and in LB films drawn from the film balance experiments. (c) Pressure/area isotherms for acidic, neutral, and basic proteins at 10^{-8} M on the Langmuir film balance.

Table 1. Basic, Neutral, and Acidic Proteins on the Langmuir Film Balance: Dependence of the Area Increase ΔA on the IEP Values at Concentrations around 10^{-8} M, Corrected for Their Individual Surface Areas

protein (10^{-8}M)	c_{cor} [M][a]	ΔA_{matrix} [A²][b]	ΔA_{rec} [A²][c]	pI	MW [kDa]	surface [kA²][d]
histone H1	4×10^{-9}	0	5	10.4	7.7	4.3
cytochrome C	6×10^{-9}	0	5	9.5	12.3	6.3
proteinase K	10^{-8}	1	6	8.1	38.4	11.0
chymotrypsin	10^{-8}	1	5	8.0	28.2	11.2
thrombin	10^{-9}	1	2	7.5	32.0	15.5
albumine	4×10^{-9}	1	2	6.0	86.3	37.0
Dps (dodecamer)	7×10^{-9}	1	1	5.9	190.0	75.0
ferritin	10^{-8}	1	2	5.5	455.3	175.5
acyl carrier protein	10^{-8}	1	1	4.2	8.4	4.7

[a] $c_{cor} = c_{exptl} \times surface_{prot}/10kA^2$. [b] Interaction with stearic acid alone. [c] Additional interaction with the embedded receptor. [d] Connolly surface.

the stearic acid monolayer. Weak to moderate effects are produced with acidic to essentially neutral proteins (+1 to 2 A²), whereas proteins with basic domains are more efficient (+2 to 3 A²). The largest shifts, however, are found with basic proteins whose pI surpasses 7 (+5 to 6 A²). All examined proteins are listed in Table 1.

A good correlation is found between the area increase in the monolayer ΔA_{rec} and the pI values of the respective proteins. Our simple model system has thus successfully mimicked the efficient and selective binding of certain proteins by multipoint recognition on cell surfaces.

That these effects truly originate from multiple cooperative recognition events and not from unspecific electrostatic attraction[17] could be proven when other anionic amphiphiles were embedded in the monolayer: even a xylylene bisphosphonate, which is moderately selective for basic amino acids, produced only very small shifts (~0.5 A²). With a monophosphate and SDS, no or negligible shifts were observed (0 to 0.5 A²). Thus, artificial receptors embedded in a monolayer were demonstrated to be capable of "multipoint binding" of complementary charged proteins, similar to the natural example.

A valuable tool to identify those protein surface areas suitable for interaction with the tetraphosphonate receptor is the electrostatic potential surface (EPS). In Figure 2a, cytochrome C is depicted with a Connolly surface[18] and the typical EPS color code (blue = positive charge, red = negative charge). Apparently, those proteins with a large flat surface area covered with arginines and lysines are optimal binding partners for the lipid/tetraphosphonate monolayer. If only one such domain exists, the protein molecules must be oriented parallel to each other to dock on to the anionic binding sites on the monolayer "ceiling". This might facilitate their crystallization.[19] We will carry out experiments along these lines soon.

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Supporting Information Available: Synthetic details, NMR binding experiments, Langmuir film experiments, and molecular modeling data. This material is available free of charge via the Internet at http://pubs.acs.org.

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