Photocontrol of Protein Conformation in a Langmuir Monolayer

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We report a method to control the conformation of a weak polyampholyte (the protein β-casein) in Langmuir monolayers by light, even though the protein is not photosensitive. Our approach is to couple the monolayer state to a photochemical reaction excited in the liquid subphase. The conformational transition of the protein molecule is triggered through its sensitivity to a subphase bulk field (pH in this study), changing in the course of the photochemical process. Thus, reaction of photoaquation of the ferrocyanide ion, which increases the subphase pH from 7.0 to about 8.3, produces a change in the surface monolayer pressure, $\Delta\Pi$, between -0.5 and +1.5 mN/m (depending on the surface concentration), signalling a conformational switch. The approach proposed here can be used to selectively target and influence different interfacial properties by light, without embedding photosensitisers in the matrix.

INTRODUCTION

It is well known that the state of monolayers spread on a liquid support can be changed by light. So far, the approach has been to prepare Langmuir monolayers of specially synthesised photochromic substances able to isomerise under illumination with light of a suitable wavelength. Light-induced *cis-trans* isomerisation of chromophores based on azobenzene and stilbene has often been used [1, 2, 3, 4, 5] and large changes in the monolayer pressure-area isotherms have been achieved [2]. Another extensively exploited isomerisation reaction is based on the photoconversion of spiropyran chromophores into merocyanine species [6, 7, 8, 9, 10]. In this paper we propose a different approach, where the properties of the bulk liquid subphase are changed by a photochemical reaction. Using the sensitivity of the monolayer molecules to changes in the subphase, an indirect photoresponse is triggered in the monolayer. Thus, it is not necessary to chemically modify the monolayer in order to achieve a photocontrolled response. Following this approach, we demonstrate here how the rich conformational behaviour of a protein in a Langmuir monolayer can be controlled and directed non-invasively by light.

PHOTOCHEMISTRY

We used the photoaquation [11] of hexacyanoferrate (II) ion, $Fe(CN)_6^{4-}$, to change the pH of the liquid subphase beneath a β-casein monolayer. Under illumination in aqueous solution, one of the cyanide ions is released from the co-ordination sphere of Fe^{2+} and substituted by a water molecule to give aquapentacyanoferrate (II), $Fe(CN)_5H_2O^{3-}$. The released CN^- is protonated to the weak hydrocyanic acid, HCN, which causes a pH increase in the solution [12]. These processes are reversible when the illumination is stopped. In this way, ferrocyanide photoaquation may be used to photochemically control pH. Recently, a similar concept was used in order to tune the effective spontaneous curvature of giant phospholipid vesicles [13].

We measured photometrically the light-induced pH change of 1 mM potassium hexacianoferrate (II) solution. Upon irradiation,the pH increases monotonically from 7.0 to about 8.3. After turning off the light, the pH decreases to a constant value, slightly higher (by about 0.1) than the initial "dark" pH. It has been shown [12] that a number of additional chemical processes can be expected (e.g., dimerisation of $Fe(CN)_5H_2O^{3-}$, acid-base equilibrium of the dimer, etc.) which have resulted in a non-monotonic pH change with time. These, however, take place at longer illumination times and slightly higher concentrations. The monotonic pH increase during illumination, observed by us, is a strong indication that the ferrocyanide aquation and cyanide protonation are the primary photochemical processes under our experimental conditions.

PROTEIN MONOLAYER CHARACTERISATION

The protein β-casein is a weak polyampholyte with an amino acid sequence with basic and acidic groups, whose charge can be adjusted by pH. Its isoelectric point pI $\approx$ 5. The N-terminal region is more hydrophilic than the rest of the chain, because many of the groups bear charge [14]. When the protein is spread as a Langmuir monolayer, this hydrophilic part would be expected to extend into the subphase to form a "tail" [15, 16, 17].

We characterised the protein monolayer by recording the surface pressure - surface concentration isotherms, $\Pi(\Gamma)$, using a Langmuir trough. Figure 1a shows the results for two different subphase pH values, 7.0 and 8.3, corresponding to the expected change in the pH due to the photochemical reaction. Figure 1b represents the static dilational modulus, $\epsilon = \Gamma(d\Pi/d\Gamma)$, as a function of Π, extracted from Figure 1a. At low surface coverage, $\Gamma < 0.8$ mg/m^2, the protein molecules are assumed to be adsorbed entirely on the liquid surface in train conformation with no tails or loops protruding into the liquid phase. Here, the surface pressure at pH = 8.3 is

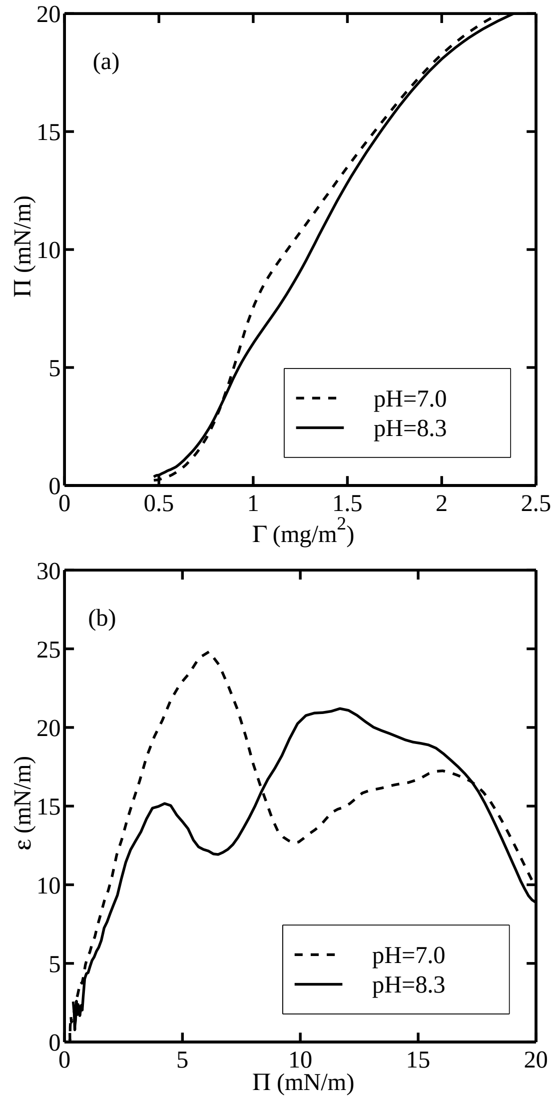

FIG. 1: (a) Surface pressure-surface concentration isotherms of β-casein monolayer at pH = 7 (dashed line) and pH = 8.3 (solid line). The subphase pH was adjusted by 100 mM phosphate buffer. (b) Static dilational modulus, ϵ, as a function of the surface pressure, Π, as calculated from Figure 1(a).

slightly, but distinguishably higher than the surface pressure at neutral pH (see Figure 1a). This reflects the fact that the polyampholyte molecule is more swollen at higher pH due to increased charge density. Conformational changes, such as tail protrusion in the liquid and/or formation of loops, are expected when the monolayer is compressed, depending on the subphase pH and ionic strength [15, 16, 18]. It has been proposed that a maximum in the ϵ *vs.* Π dependence (Figure 1b) could be considered as an onset of a conformational transition and the following minimum is the end of that transition [15, 18, 19]. On this basis, the first maximum (at $\Pi \approx 6$ mN/m for pH = 7 and $\Pi \approx 4$ mN/m for pH = 8.3) appears to reflect the onset of tail formation, due to the more hydrophilic N-terminus of the β-casein molecule [18, 19]. The second maximum, at $\Pi \approx 16$ mN/m for pH = 7 and $\Pi \approx 11$ mN/m for pH = 8.3, has been tentatively assigned to the onset of loop formation [18]. Direct structural probe techniques to resolve the surface conformation of this protein, like neutron [20] or X-ray reflectivity, have been limited to high surface concentrations. Only recently, Harzallah et al. [21] were able to achieve enough sensitivity at relatively low surface coverage between 1.14 and 2.69 mg/m^2 for adsorption layers of β-casein. At the lowest $\Gamma = 1.14$ mg/m^2 and pH = 7.1, the molecules were accomodated on the liquid surface as trains (56 %) and loops or the N-tail (44 %) with area per molecule ≈ 3500 Å^2. In contrast, at higher concentrations ($\Gamma > 2.13$ mg/m^2), the train fraction decreased to about 30 %, and the rest 70 % of the protein sequence was present as loops and long tails. This, together with a two-fold decrease in the area per molecule to ≈ 1870 Å^2, is in unison with the simplified conformational picture conjectured on the basis of thermodinamic (Π, ϵ) measurements. A detailed discussion of the conformational behaviour in β-casein monolayers can be found in [18].

PHOTOCONTROL OF PROTEIN CONFORMATION

The experimental set up to induce conformational transitions of the protein monolayer is straightforward. We chose to work with small surface areas in order to achieve more intense and homogeneous illumination and used a small circular vessel (Petri dish of 6.8 cm diameter) as the Langmuir trough. The light source, a 100 W halogen lamp, was mounted about 15 cm from the liquid surface to produce unattenuated homogeneous illumination. The power density, measured using a calibrated photodiode after a 505-575 nm bandpass filter, was 18 mW/cm^2. The protein monolayer was spread on a subphase containing 1 mM potassium hexacyanoferrate (II) and 100 mM NaCl. The surface pressure was recorded by a Wilhelmy plate. For each experiment, a new monolayer of different surface concentration was spread, which was equivalent to the standard Langmuir trough procedure, where the surface concentration is altered by changing the surface area [22]. This procedure also ensured a standard initial state of the monolayer under “dark” conditions. After recording the surface pressure, the photochemical reaction was initiated by turning the illumination on and the surface pressure relaxation was followed.

We recorded light-induced jumps in the surface pressure spanning the entire region of surface concentrations from 0 to about 2.2 mg/m^2. Four examples, for different concentration regions, are shown in Figure 2. Generally, we observed fast changes in the surface pressure before it levelled off to a plateau (see Figure 2). Often, especially at higher surface pressures, the system exhibited a more

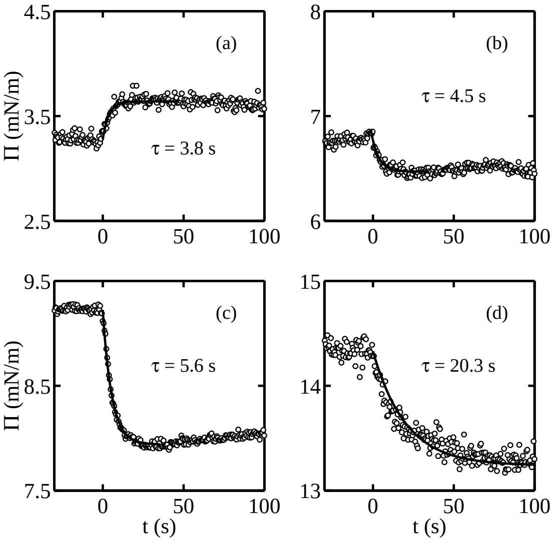

FIG. 2: Four examples of light-induced surface pressure jumps for different initial surface concentrations, signalling different types of conformational switch. The surface concentration and the corresponding initial surface pressure increase from (a) to (d). The relaxation times τ were extracted by exponential fits (solid lines) to the experimental data.

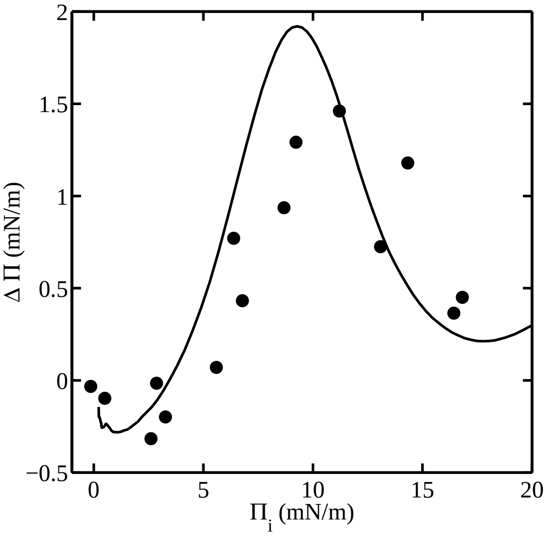

FIG. 3: Dependence of photoinduced surface pressure jump, $\Delta\Pi = \Pi_i - \Pi_f$, on the initial monolayer surface pressure in "dark", Π_i (solid circles). The solid line represents the difference in the monolayer surface pressures for two different values of the subphase pH, 7.0 and 8.3, calculated from Figure 1a.

complicated pressure trend over a longer time scale (data not shown) most probably due to stress redistribution in the viscoelastic protein network.

Two additional control experiments were performed. First, we checked the impact of the heat produced by the light source in a system without the photochemical compound. This produced a negligible change in the surface pressure. The second experiment was performed in the presence of ferrocyanide, but this time in a pH buffered subphase, ensuring that although the photochemical reaction occurred, there was no change in pH. Again, no change in the surface pressure was observed during illumination. This is an evidence that the primary photochemical coupling is by the photo-induced pH change. Photoinduced changes in the ionic strength were negligible, compared to the ionic strength set by the inert monovalent electrolyte used throughout all the experiments (100 mM NaCl).

Figure 3 shows the dependence of the photoinduced surface pressure jump, $\Delta\Pi$, on the initial surface pressure under "dark" conditions, Π_i. The solid line shows the expected jump in the surface pressure due to the change in the subphase pH, obtained from the pressure-concentration isotherms in Figure 1. The agreement is fairly good, with the photochemical system recovering all the important features, particularly the negative value of $\Delta\Pi$ at very low initial pressures and the presence and approximate position of the maximum.

The protein behaviour can now be discussed on the basis of the proposed molecular conformation as evidenced by the change in the static dilational modulus due to pH changes (see Figure 1b and its discussion above). At low surface concentrations (corresponding to low initial surface pressures), irradiation leads to increase in the surface pressure (Figure 2a) and $\Delta\Pi$ is negative. Here, the molecules are in all-train conformation. The light-induced pH increase causes swelling of the molecules due to their charging and this leads to increased surface pressure. With the increase of the initial pressure, a crossover to positive $\Delta\Pi$ is observed and the photoinduced conformational switch has a different character. The pressure relaxations in Figures 2b and 2c reflect transitions from train to tail conformations (cf Figure 1b). That of Figure 2d can be assigned to a light-induced formation of loops, i.e., transition from tail to tail-and-loop conformation.

All data for the surface pressure relaxation are described well by a single relaxation time, τ, using the exponential decay function $\Pi(t) - \Pi_f = \Delta\Pi \exp(-t/\tau)$ (where Π_f is the plateau value of the surface pressure). The exponential relaxation in this system can be understood as a first-order kinetics assuming a rate of conformational reorientation proportional to the instantaneous number of molecules to be converted [23]. Further, we found an increase of the relaxation time with the increase of the initial pressure Π_i (Figure 4). This fact makes clear that the slowest and therefore rate-determining step in the whole chain of processes (photoinduced pH increase $\rightarrow$ charging of molecules $\rightarrow$ conformational transition) is the conformational rearrangement in the protein monolayer. Obviously, the $\tau(\Pi_i)$ trend is due to steric hindrance and

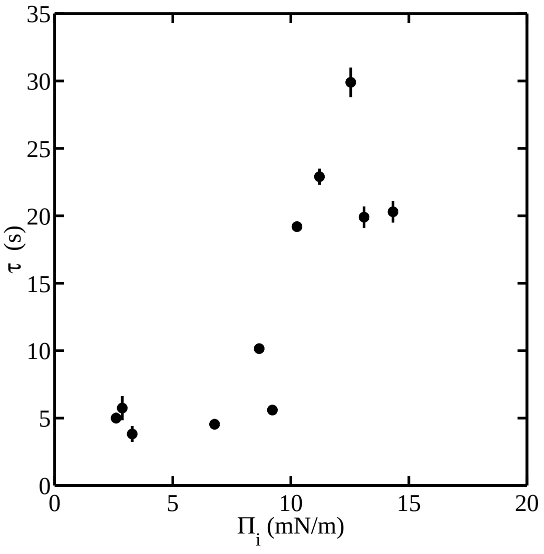

FIG. 4: Dependence of the relaxation time, τ, on the initial monolayer surface pressure in "dark", Π_i.

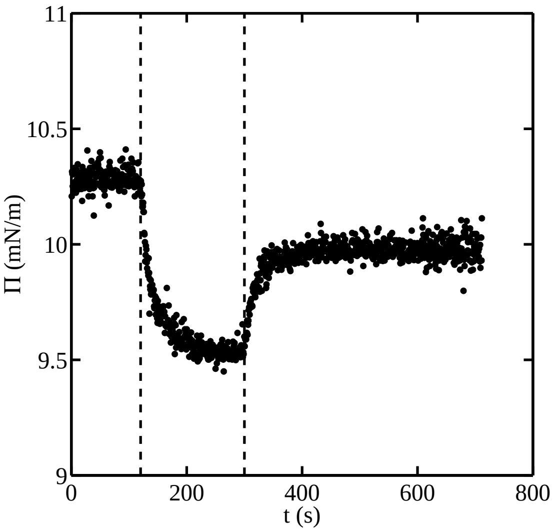

FIG. 5: Reversibility of the photo-induced conformational switch. The illumination has been turned on at $t = 120$ s and off at $t = 300$ s (see the dashed lines).

entanglement of the protein molecules at higher surface concentrations, increasing the energy barrier for conformational transition. By way of contrast, a recent study of *cis-trans* photoisomerisation kinetics in Langmuir monolayers of azobenzene-containing dendrons showed the opposite trend: the isomerisation was facilitated at higher pressures, probably due to collective behaviour [5].

Finally, we would like to discuss the reversibility of the photoinduced conformational transitions. As mentioned earlier, the ferrocyanide photoaquation is reversible upon turning the illumination off. Our experimental results show that full recovery of the surface pressure after ceasing the illumination is possible at low surface concentrations, where the only process induced is the swelling/deswelling of the polyampholyte chain. The system exhibits interesting behaviour in the intermediate region of the initial surface pressures between 5 and 8 mN/m. Light-induced transitions here are almost completely irreversible (data not shown) and the monolayer appears to be trapped in a metastable state after the illumination. This corresponds, according to Figure 1, to the only region of surface concentrations, where both the pressure and the dilational modulus must increase if the monolayer were to relax to its initial condition after the illumination has been terminated. At higher surface concentrations (initial pressures above 8 mN/m), the conformational transitions are, at best, only partially reversible, despite the practically full recovery of the initial "dark" value of the subphase pH. This situation is illustrated in Figure 5. Another factor affecting the conformational reversibility is the exposure time. Longer illumination times appear to make the light-induced conformational transition irreversible.

SUMMARY AND CONCLUSIONS

In summary, we have demonstrated a novel method to control the molecular conformation in Langmuir monolayers by coupling the monolayer state to a photochemical reaction taking place in the bulk of the liquid subphase. We have shown how different types of conformational switch (swelling of the polyampholyte chain, train-to-tail and tail-to-tail-and-loop transitions) can be induced by light by selecting the surface concentration. We consider the present approach as a particular case within a more general scheme of coupling between ongoing bulk chemistry and interfacial material properties and topology, manifested in many biological and technologically important processes. Identification of suitable couplings, especially to photochemical and oscillating chemical reactions, would be of particular interest in this respect.

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