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Interaction of nominally soluble proteins with phospholipid monolayers at the air–water interface

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Abstract

The interactions of carbonmonoxyhemoglobin (HbCO), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and polyhistidine with phospholipid monolayers at the air–water interface were studied at physiological pH and ionic strength. HbCO and GAPDH both interact more strongly with monolayers containing negatively charged lipids. The interaction of HbCO and GAPDH with lipid monolayers decreases with increasing pH. Both the HbCO–monolayer and the GAPDH–monolayer interactions can be modeled as diffusion-limited processes, with kinetic data fit to a stretched exponential equation. The significance of these kinetics are discussed. Polyhistidine interacts only with monolayers containing lipids with negatively charged headgroups. In total, the results presented are consistent with an HbCO–lipid interaction with a large electrostatic component, a GAPDH–lipid interaction with comparable electrostatic and hydrophobic components, and a polyhistidine–lipid interaction that is solely electrostatic.

Keywords: Protein–lipid interaction; Air–water interface; Hemoglobin; Glyceraldehyde-3-phosphate dehydrogenase; Phospholipid Monolayer

1. Introduction

Hemoglobin (Hb) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) have important roles in oxygen transport and glycolysis, respectively. Both of these proteins have been reported to bind to lipid monolayers (see Ref. [1] for a review). In this study the interactions of these proteins with lipid monolayers at the air–water interface are examined.

GAPDH in particular has been implicated in the maintenance of erythrocyte shape [2]. Addition of exogenous amphipaths can induce shape changes in erythrocytes, as described by the bilayer couple hypothesis [3]. In membrane systems with lipid asymmetry, such as the human erythrocyte [4], molecules that preferentially intercalate in the inner/outer monolayer of the membrane increase the inner/outer surface area of the membrane and cause an inward/outward buckling of the membrane. The postulated role of GAPDH in erythrocyte shape control is as an inner monolayer intercalator to counteract exogenous outer monolayer intercalators. GAPDH, with dual roles of glycolysis and shape control, is an example of a moonlighting protein. Many examples of moonlighting proteins have been discovered [5,6]. This study examines the GAPDH–lipid interaction.

Traditionally proteins have been thought of either as globular and soluble or membrane-bound and insoluble. This study will show that even nominally soluble, cytosolic proteins, like Hb and GAPDH, can interact with lipid monolayers and thus cell membranes. At times, the interaction between cytosolic proteins and lipids is strong and may have a physiological role.

The interaction of polyhistidine with lipid monolayers is also examined. Histidine-rich proteins may be involved in the fusion of infectious organisms with mammalian cells. For example, the malaria parasite produces a histidine-rich protein (although inoculation of hosts with this histidine-rich protein confers protection from malarial infection) [7].

The present studies use surface pressure measurements of phospholipid monolayers at the air–water interface in the presence of protein [carbonmonoxyhemoglobin (HbCO), GAPDH, or polyhistidine] in the subphase. The lipid monolayer at the air–water interface serves as a model for half of a cell membrane and the subphase can be held at physiological conditions of pH and ionic strength. A wide

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variety of lipids and proteins can be examined. These features make monolayer studies a powerful tool in understanding protein–lipid interactions.

2. Materials and methods

HbCO was prepared following the method described by Moxness [8]. Carbon monoxide was bubbled through blood drawn from healthy human volunteers. After carbonmonoxylation, the blood cells were washed and lysed and the cell debris removed by centrifugation. The resulting supernatant was dialyzed against column buffer (50 mM Tris, pH 8.3) and purified on an anion exchange column (DEAE Sephadex A-50). A buffered pH gradient (pH 8.3 to pH 7.0) was run through the column. Fractions containing HbCO were combined and concentrated by ultrafiltration. Final concentration was approximately 10 mM in heme (2.5 mM protein). After ultrafiltration the methemoglobin concentration was less than 2% of total Hb concentration, as determined by multicomponent UV–Vis analysis. HbCO aliquots (300 μl each) were stored in liquid nitrogen. HbCO was injected at a final concentration of 167 nM protein, equivalent to 667 nM in heme, approximating the physiological lipid/heme ratio in erythrocytes. GAPDH (lyophilized, from rabbit muscle) was from Sigma Chemical Co. (St. Louis, MO). Purity of GAPDH was checked by SDS-PAGE and activity was confirmed by colorimetric assay [9]. GAPDH stock solutions were prepared by dissolving GAPDH in buffer [5 mM phosphate or phosphate-buffered saline (PBS)] and adjusting the pH. Poly-L-histidine (polyhistidine) was from Sigma and had an average molecular weight of 15,800. Polyhistidine stock solutions were prepared by dissolving polyhistidine in nanopure water. Protein was added to the subphase either by pre-mixing prior to monolayer formation or by injection beneath the monolayer without mixing. Pre-mixing was used in short timescale (less than 15 min) experiments, while injection was used in longer timescale (several hours) experiments. For injection experiments, monolayer surface pressure was allowed to stabilize before addition of protein (approximately 1 min).

Lipids [dimyristoylethylphosphatidylcholine (DMetPC), dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylglycerol (DMPG), dimyristoylphosphatidylserine (DMPS, Avanti Polar Lipids, Alabaster, AL), and dihydrocholesterol (Dchol, Sigma)] were dissolved in chloroform. Phospholipids were chosen to have approximately the same chain length and to be in a liquid phase at the surface pressures studied. For binary lipids mixtures (DMPC/DMPS, DMPC/DMPG, DMPC/DMetPC), the molar ratio was 1:1. Total lipid concentrations in these stock solutions were approximately 2 mg/ml. Stock solutions of lipids were diluted 10:1 before deposition on the air–water surface of a homebuilt teflon Langmuir trough, which was cleaned with chloroform and rinsed with water after each experiment. Monolayer surface pressure at room temperature (21 °C) was measured by a Wilhelmy plate of filter paper [10] and recorded on a chart recorder. Surface pressure values begin at 0 mN/m for a bare air–water interface and increase to a maximum of 72 mN/m. Lipids were added until an initial monolayer pressure of approximately 1 mN/m was obtained. Although 1 mN/m is too low for the lipids to structurally mimic a cell membrane, the primary goal of these experiments was to measure the effect of lipid charge on surface activity of the proteins. Fits of the data were performed using KaleidaGraph 3.0 software.

Solution viscosity was adjusted by addition of dextran (dextran T-70, average molecular weight 70,000). Viscosities were measured in a Cannon-Fenske viscometer (no. 100 size) according to the method of Shoemaker et al. [11–13]. Aqueous dextran solutions with pH ≤ 7.0 (below the pI of Hb) were used without additional buffering.

3. Results

3.1. HbCO shows increased surface activity in the presence of anionic lipids

Since HbCO is a charged protein, it is reasonable to expect that its surface activity is affected by the charge of the lipids in the monolayer. Even in the absence of lipids, injection of HbCO into the aqueous subphase of the trough produces an increase in surface pressure from 0 to 22 mN/m, as seen in Fig. 1. In the presence of neutral lipids only (DMPC), injecting HbCO caused the surface pressure to increase at a similar rate from 2 to 26 mN/m (Δπ, 24 mN/m). In the presence of cationic lipids (DMPC/DMetPC), HbCO injection resulted in a slower increase in surface

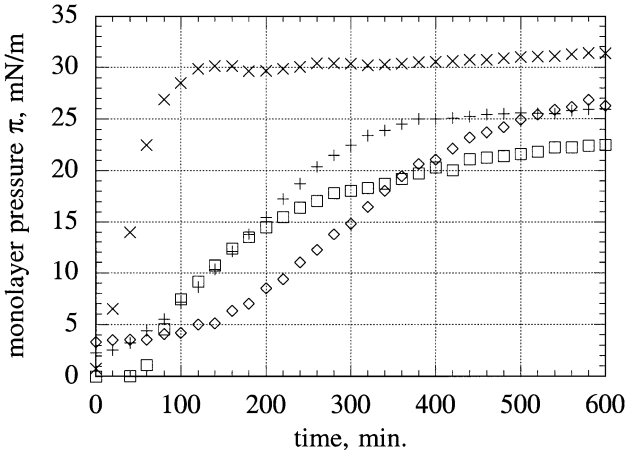

Fig. 1. Monolayer pressure, π, vs. time, HbCO injected into water subphase (final protein concentration 167 nM). Data are for different monolayer compositions: (×) DMPC/DMPS; (+) DMPC; (◇) DMPC/DMetPC; (□) no lipids present.

pressure from 4 to 26 mN/m (onset after approximately 2 h) but a similar $\Delta\pi$, 22 mN/m. With anionic lipids present (DMPC/DMPS), HbCO injection resulted an increase in surface pressure that was both faster and larger (from 0 to 30 mN/m, $\Delta\pi = 30$ mN/m). On this timescale (10 h), change in monolayer surface pressure due to evaporation was not observed. On a timescale of less than 15 min, HbCO showed surface activity only when anionic lipids were present (DMPC/DMPS or DMPC/DMPG).

3.2. Effect of solution ionic strength and pH on surface pressure (HBCO)

The contribution of HbCO to the surface pressure of a monolayer containing DMPC/DMPS/Dchol (3:3:2) decreases with ionic strength of subphase, as shown in Fig. 2. Fig. 3 illustrates that HbCO increased surface activity at low pH and decreased surface activity at high pH.

3.3. Kinetics of increasing surface pressure (HBCO)

Values of surface pressure vs. time were fit to the following stretched exponential equation:

$$\pi_t = \pi_\infty - \pi_0 e^{-(kt)^\beta} \quad (1)$$

where π_t is the monolayer pressure at time t, π_∞ is the equilibrium monolayer pressure, π_0 is the initial monolayer pressure, k is the rate coefficient, and β is an exponential scaling factor. The choice of equations is explained in the discussion section. For monolayers containing HbCO, Figs. 4 and 5 show the relationship between pH of a subphase containing 5 mM phosphate and either k or β, respectively.

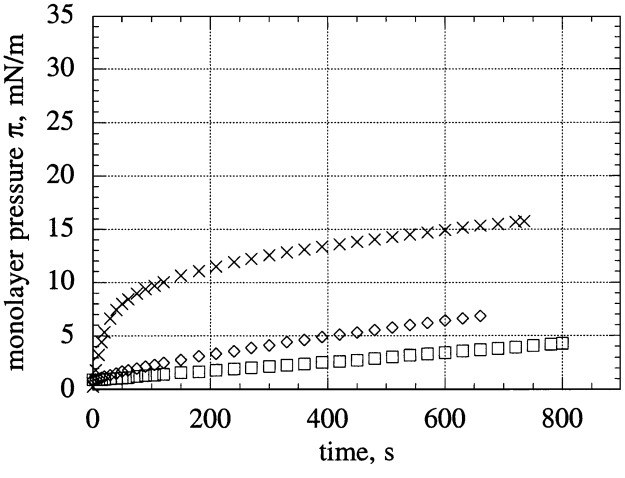

Fig. 2. Monolayer pressure, π, vs. time, 167 nM HbCO subphase, DMPC/DMPS/DChol (3:3:2) monolayer. Data are for different subphase ionic strengths: (×) water; (◇) 5 mM phosphate; (□) PBS. Rate coefficients were found to be 0.0132 ± 0.0032, $0.00213 \pm 1.9e-4$, and $7.07e-4 \pm 1.8e-4$ s^{-1} for water, 5 mM phosphate, and PBS, respectively.

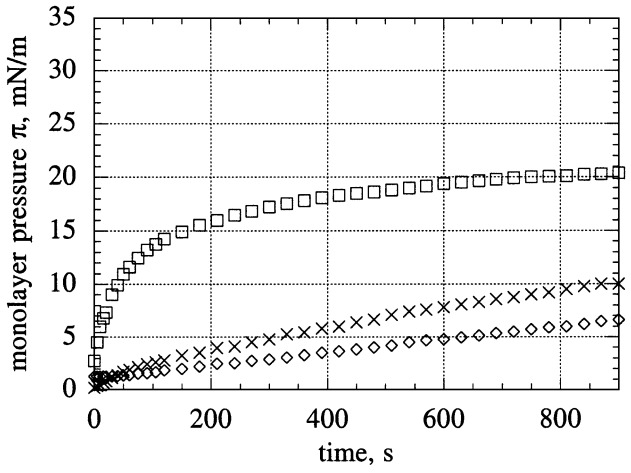

Fig. 3. Monolayer pressure, π, vs. time, 167 nM HbCO in 5 mM phosphate buffer subphase, DMPC/DMPS (50:50) monolayer. Data are for different subphase pH values: (□) pH 6.4; (×) pH 7.4; (◇) pH 8.4.

3.4. Effect of solution viscosity on surface pressure (HBCO)

If the transport of HbCO is diffusion-limited, it is reasonable to expect that the rate of increase of surface pressure is affected by the addition of dextran to alter the viscosity of the subphase solution. Dextran had no surface activity at concentrations less than 5 mg/ml (viscosity less than 1.12 Cp). Higher dextran concentrations of 10–100 mg/ml (viscosities 1.25–3.0 Cp) had no surface activity at short time scales of less than 15 min (data not shown). Rate coefficients were fit to a power curve (data not shown).

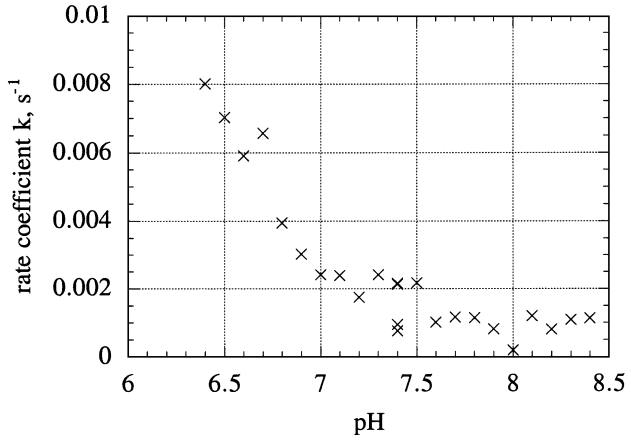

Fig. 4. Rate coefficient, k, vs. pH, 167 nM HbCO in 5 mM phosphate buffer subphase, DMPC/DMPS (50:50) monolayer.

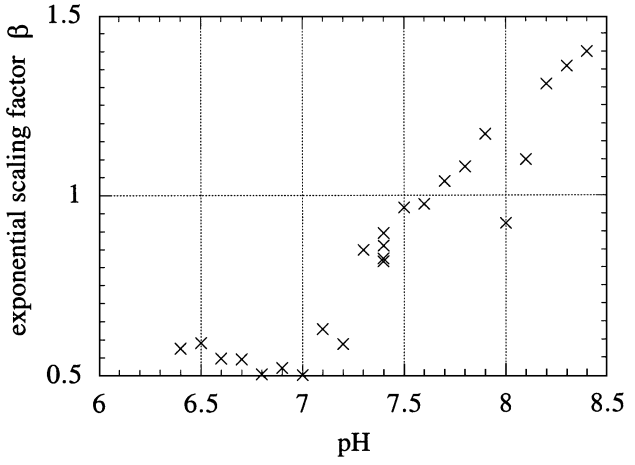

Fig. 5. β vs. pH, 167 nM HbCO in 5 mM phosphate buffer subphase, DMPC/DMPS (50:50) monolayer.

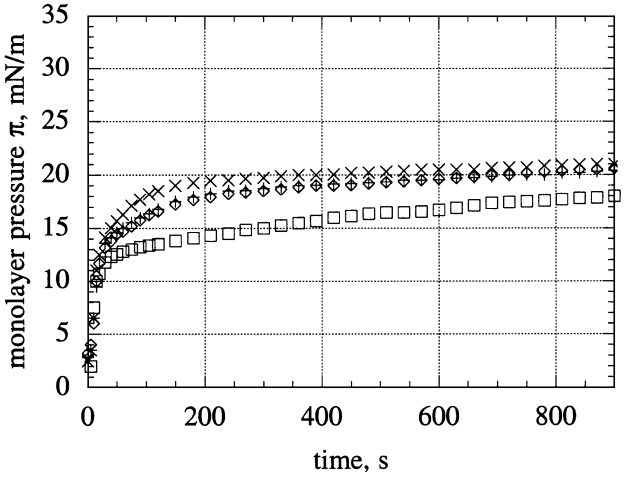

Fig. 7. Monolayer pressure, π, vs. time, GAPDH injected into PBS subphase (final protein concentration 167 nM). Data are for different monolayer compositions: (×) DMPC/DMPS; (+) DMPC; (◇) DMPC/DMetPC; (□) no lipids present.

3.5. GAPDH surface activity in the presence/absence of lipids

As with HbCO, the injection of GAPDH (final concentration of 167nM) into the aqueous subphase of the trough produces an increase in surface pressure from 0 to 22 mN/m even in the absence of lipids, as shown in Fig. 6. Addition of cationic lipids (DMPC/DMetPC), unchanged lipids (DMPC), and anionic lipids (DMPC/DMPS) results in successively higher values of surface pressure ($\Delta\pi = 21$, 25, and 27 mN/m, respectively) and generally faster increases.

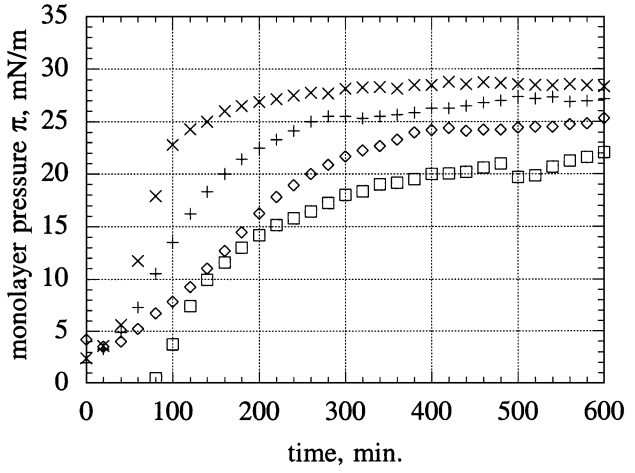

Fig. 6. Monolayer pressure, π, vs. time, GAPDH injected into 5 mM phosphate buffer subphase (final protein concentration 167 nM). Data are for different monolayer compositions: (×) DMPC/DMPS; (+) DMPC; (◇) DMPC/DMetPC; (□) no lipids present.

3.6. Effects of ionic strength and pH on GAPDH–monolayer interaction

In systems of higher subphase ionic strength (PBS, 150 mM salt), injection of GAPDH into the subphase resulted in immediate surface activity. For all systems, with or without lipids present, $\Delta\pi$ was 18 mN/m. Systems without lipids, with neutral lipids (DMPC), or with cationic lipids (DMPC/DMetPC) had rate coefficients of approximately 0.022 s^{-1}. Systems with anionic lipids (DMPC/DMPS) had a faster increase in surface pressure, with a rate coefficient of 0.031 s^{-1}. These results are illustrated in Fig. 7 and summarized in Table 1.

Injection of GAPDH into a PBS subphase of a DMPC/DMPS monolayer resulted in the same $\Delta\pi$, 18 mN/m, whether of the subphase pH 6.4, 7.4, or 8.4 (Fig. 8). The rate coefficient for the GAPDH–monolayer interaction was also the same (within errors for the fits) for all three subphase pH values.

3.7. Kinetics

Data for the increase in surface pressure due to GAPDH vs. time were fit to the same equation (Eq. (1))

Table 1
Rate coefficients and β values for fit of surface pressure vs. time of GAPDH in PBS subphase

Monolayer lipid composition	k (s^{-1})	β
No lipids	0.021 ± 0.004	0.42 ± 0.04
DMPC	0.022 ± 0.001	0.61 ± 0.03
DMPC/DMPS	0.031 ± 0.002	0.71 ± 0.04
DMPC/DMetPC	0.023 ± 0.002	0.62 ± 0.03

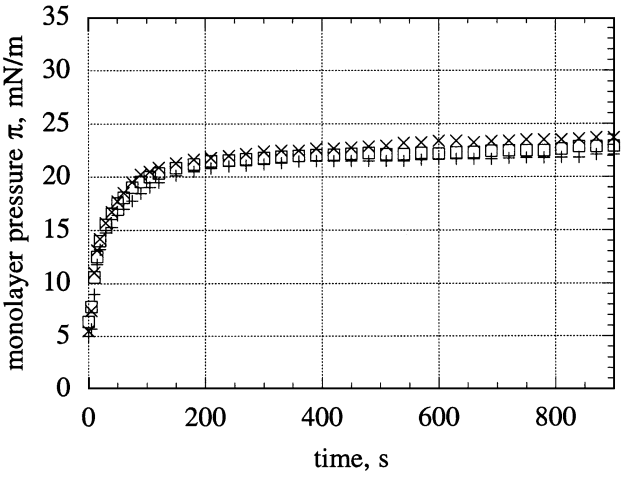

Fig. 8. Monolayer pressure, π, vs. time, GAPDH injected into PBS subphase (final protein concentration 167 nM), DMPC/DMPS (50:50) monolayer. Data are for subphase pH values: (×) pH 6.4; (+) pH 7.4; (□) pH 8.4.

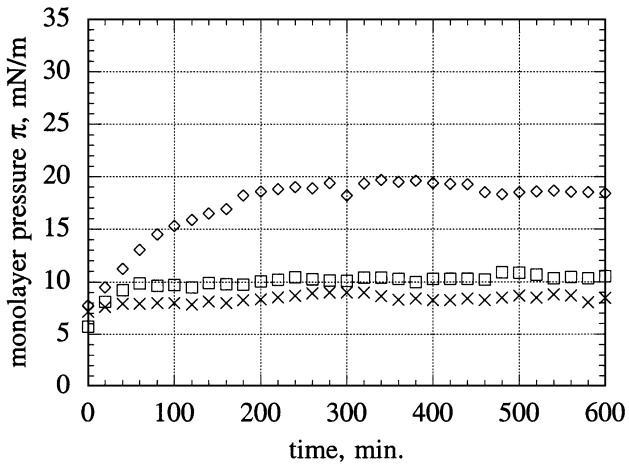

Fig. 10. Monolayer pressure, π, vs. time, polyhistidine injected into PBS subphase (final protein concentration 5 μM), DMPC/DMPS (50:50) monolayer. Data are for subphase pH values: (◇) pH 6.4; (□) pH 7.4; (×) pH 8.4.

as data for HbCO. Rate coefficients and β values are shown in Table 1.

3.8. Surface activity of polyhistidine

Polyhistidine (poly-L-histidine, average molecular weight 15,800) injected into the subphase at a final concentration of 5 μM does not show surface activity in the absence of lipids at the air–water interface. When anionic lipids (DMPC/DMPS) are deposited on the surface, surface pressure increases. Figs. 9 and 10 illustrate this for subphases containing low and high ionic concentrations, respectively. At low ionic strength (5 mM phosphate buffer), and pH 7.4 or 8.4, surface pressure increased from approximately 5 to 9 mN/m over the course of hours. At lower pH of 6.4 surface pressure increases further from approximately 8 to 16 mN/m over several hours. At high ionic strength (PBS), the situation is nearly repeated except that at pH 6.4, a higher surface pressure (19 mN/m) is reached in a shorter time.

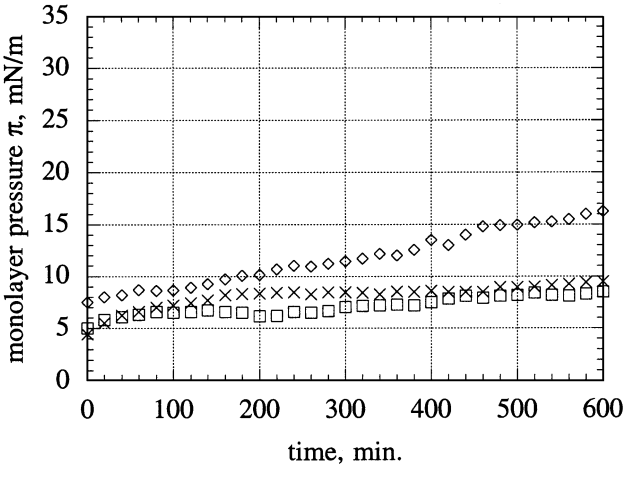

Fig. 9. Monolayer pressure, π, vs. time, polyhistidine injected into 5 mM phosphate buffer subphase (final protein concentration 5 μM), DMPC/DMPS (50:50) monolayer. Data are for subphase pH values: (◇) pH 6.4; (□) pH 7.4; (×) pH 8.4.

4. Discussion

Studies of HbCO with lipid monolayers at the air–water interface indicate that the interaction between HbCO and surface is primarily electrostatic. Although HbCO shows surface activity when no lipids are present, it shows the greatest surface activity in the presence of phosphatidylserine (PS), which is negatively charged. Surface activity is observed on timescales of less than 15 min only when PS is present in the monolayer. Furthermore, HbCO surface activity decreases with increasing subphase ionic strength. Increasing ionic strength from 0 mM (water) to 5 mM results in a fivefold decrease in rate, while increasing ionic strength to 150 mM (PBS) results in a further threefold decrease in rate. The increase in surface activity of HbCO with decreasing pH is likely due to the titration of amino acid residues (namely histidine) in HbCO. The aqueous phase isoelectric point of Hb is 7.1. Fig. 4 shows a significant change in rate of monolayer interaction around that pH. The best fit (coefficient of determination, $R^2 = 1.0$) could be obtained only by fitting two lines to the data; a line fit through the slope of the data (rate coefficient vs. pH) yields a slope of -8.8×10^{-3} for pH values less than 7.2, while for pH values greater than 7.4 the slope is an order of magnitude smaller (slope $= -6.8 \times 10^{-4}$). Similar behavior is seen in Fig. 5, where β is in the range of 0.5–0.6 for pH values less than 7.3 and is 0.9–1.4 for pH values of 7.3 and

greater. Below the isoelectric point, HbCO has a net positive charge, making electrostatic interactions with the negatively charged PS headgroup probable. Even above the p*I*, where HbCO should have a net negative charge, some amino acid residues may still carry positive charges. These findings are consistent with reports of other proteins shown to interact with lipid monolayers in a predominantly electrostatic manner, including myelin β-lactoglobulin [14] and GM2-activator protein [15].

The rate constants extracted from the fit of surface pressure vs. time to a stretched exponential equation (Eq. (1)) can be used to learn about the process by which HbCO approaches the air–water interface. An exponential scaling factor $\beta = 0.5$ is indicative of a diffusion-limited process. In the protein–lipid monolayer system, such kinetics would describe a protein molecule undergoing a random walk through the subphase with some fraction of walks ending at the air–water interface. When $\beta = 1$, first-order kinetics are obtained. Values of β greater than 1 do not correspond to known physical processes and it is unknown why values of $\beta > 1$ are found [16,17].

Stretched exponential kinetics have been studied previously for various model systems of molecules at surfaces [18,19] including uncharged polymers at solid–liquid and liquid–liquid interfaces [20,21], polyelectrolytes at the solid–liquid interface [22,23], and proteins at the solid–liquid interface [24,25]. Stretched exponential kinetics have also been used to describe chemical and physical rate processes in disordered media, including protein–ligand interactions, reaction kinetics of biopolymers, and the statistical distributions of open and closed times of ion channels [17]. Diffusion-limited reactions describe a wide variety of systems, even those involving charged polymers (or proteins) and charged surfaces.

If the transport of HbCO to the lipid surface is diffusion-limited, the rate coefficient, k, should be proportional to the diffusion coefficient of HbCO and thus, according to the Stokes–Einstein equation [26], inversely proportional to the viscosity of the subphase such that $k = c/\eta$. This prediction was tested by altering subphase viscosity, η, via addition of dextran. Microscopic viscosity (on the scale of HbCO molecules) was assumed to be the same as macroscopic viscosity, since the experiment used dextran with an average molecular weight of 70,000, which is approximately the same size as HbCO. Data fit to a power curve showed an inverse relationship between rate coefficient and viscosity, though scatter in the data prohibited quantitation of this inverse relationship (i.e., fits ranging from $k = c/\eta$ to $k = c/\eta^4$ could not be differentiated).

The surface activity of GAPDH is less dependent on electrostatic interactions than HbCO, as shown. The rate of surface pressure increase due to GAPDH was slightly higher when the lipid monolayer contained negatively charged PS. However, Fig. 8 shows that a change in pH from 6.4 to 8.4 does not affect the rate of increase of surface pressure. Using subphase of PBS rather than low ionic strength 5 mM phosphate, the final surface pressure was independent of which lipids were in the monolayer. Also, the rate of surface pressure increase was much faster with PBS. This could be due to a ‘‘salting-out’’ effect such that in an increasingly ionic environment the largely hydrophobic GAPDH goes to the hydrophobic air–water interface. This result is in contrast to that observed with HbCO, where the presence of additional ionic species in solution screens the protein–lipid interaction. The combination of these results indicates that although there is an electrostatic component to the GAPDH–lipid interaction, there is also a significant hydrophobic component. Studies of other protein–monolayer systems suggest that the combined electrostatic and hydrophobic interaction is reasonable [27–30].

The pressure–time data for the GAPDH–lipid interaction were fit to the same stretched exponential equation (Eq. (1)) as the data for the HbCO–lipid interaction. As Table 1 shows, β is slightly higher (0.71 ± 0.04) at DMPC/DMPS monolayer vs. other lipid monolayer systems. The DMPC/DMPS system may deviate more from the diffusion-limited model ($\beta = 0.5$) due to electrostatic interactions between the PS headgroup and GAPDH. When no lipids are present ($\beta = 0.42 \pm 0.04$) the system behaves closest to the diffusion-limited model of $\beta = 0.5$.

The polyhistidine–lipid monolayer interaction is almost entirely electrostatic. In the absence of a lipid monolayer, polyhistidine shows no surface activity, even at protein concentrations 30 times that used for the HbCO and GAPDH studies. Even in the presence of a DMPC/DMPS monolayer, there was little increase in surface pressure. Only at pH 6.4, where polyhistidine would have a larger positive charge, does the surface pressure increase more than 4 mN/m. This result is consistent with previous studies of other polypeptides [31,32]. Despite the results above, increasing salt concentration does not diminish the observed surface pressure as expected for an electrostatic interaction and as seen previously with HbCO. There is likely a small hydrophobic component to the polyhistidine–lipid interaction, even though electrostatic forces dominate.

In summary, the basic studies presented here provide insight into the relative weight of electrostatic vs. hydrophobic interactions of nominally soluble proteins with lipid monolayers at the air–water interface, though more sophisticated studies are needed to understand all aspects of the protein–lipid interactions. The results presented here show that HbCO has a largely electrostatic interaction with lipid monolayers that increases with decreasing pH and can be screened by increasing ionic strength of the subphase. In contrast, the GAPDH–lipid monolayer interaction is largely hydrophobic rather than electrostatic. Although GAPDH has the greatest interaction with PC/PS monolayers, the difference compared to other monolayer compositions is not as great as with HbCO. With PBS in the subphase, monolayer lipid composition and pH do not affect the change in surface pressure, $\Delta\pi$. Polyhistidine is more like HbCO in that there is a primarily electrostatic component in its interaction with

lipid monolayers, as seen in the pH dependence of the interaction. The hydrophobic component of the polyhistidine–lipid monolayer interaction is likely small, as (unlike HbCO or GAPDH) polyhistidine does not show surface activity in the absence of lipids.

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