Influence of Lyotropic Liquid Crystals on the Ability of Antibodies To Bind to Surface-Immobilized Antigens

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We report an experimental study of the influence of lyotropic liquid crystalline phases on the ability of antibodies to bind to protein antigens immobilized on surfaces when the antibodies are delivered and bind to the antigens from the lyotropic liquid crystals (LCs). The LCs were prepared from anionic amphiphiles (sodium decyl sulfate (SDeS), cesium pentadecafluorooctanoate (CsPFO), and potassium laurate (KL)), cationic amphiphiles (decylammonium chloride (DACl) and cetylpyridinium chloride (CPCl)), nonionic amphiphiles (Brij 30 and Triton X114), and the zwitterionic surfactant tetradecyldimethyl-amineoxide (C14AO). In addition, we investigated LCs prepared from the nonamphiphilic molecules disodium cromoglycate (DSCG) and the dye molecule Direct Blue 67. Antihuman IgGs conjugated with the fluorescent label fluorescein-isothiocyanate (FITC) were dissolved in 12 lyotropic LCs and then incubated with human IgG that was arrayed on surfaces. Fluoresence microscopy was used to image antihuman IgG that bound to the immobilized human IgG. From this survey of lyotropic LCs, we identified three LCs (DSCG, C14AO, and CPCL) that permitted antihuman IgG dissolved in the LC to recognize and bind to surface-bound human IgG. Antibody binding in the other LCs was prevented by a number of mechanisms, including denaturation of the IgG (anionic surfactants) as well as slow mass transport due to high viscosity of the LC phases (nonionic surfactants). Because the lyotropic LC formed from DSCG exhibited the highest birefringence and the lowest viscosity among the LCs formed from DSCG, C14AO, and CPCL, the binding of antibodies in DSCG-based LCs to virus-infected cells was also investigated. We found that the binding of antibodies to vesicular stomatitis virus (VSV) that was inoculated into human epitheloid cervical carcinoma cells was highly specific in the lyotropic LC prepared from DSCG.

Introduction

The ordering of liquid crystals (LCs) near surfaces has long been known to be influenced by the chemical functionality and topography of interfaces.[1−8] Recent studies have exploited this so-called orientational anchoring of liquid crystals to provide the basis of methods that can be used to amplify and optically transduce the presence of biological molecules and their assemblies (such as viruses and cells) at surfaces.[9−13] In particular, the orientations assumed by both lyotropic[13] and thermotropic liquid crystals[9,11,12] on surfaces that are decorated with proteins have been the subject of a number of past studies. In these past studies, the liquid crystals were applied to surfaces after proteins were captured by receptors presented by the surface[9,11−13] and the issue of binding activity of the proteins *within the LC* was not addressed. In this paper, we report a study that sought to identify LCs with properties that would permit specific binding events involving proteins to occur within them. Such liquid crystalline phases may enable the use of LCs to report a number of protein functions (e.g., binding activity, enzymatic activity, or motor protein functions).

This paper is focused on the lyotropic class of LCs because the aqueous content of some lyotropic liquid crystalline phases makes them likely to be compatible with proteins. For a lyotropic liquid crystal comprised of surfactants or water-soluble mesogens to permit specific binding events to occur between proteins, we hypothesized that the LC must possess several properties. First, the lyotropic liquid crystal must not denature the protein structure to an extent that results in loss of specific binding activity. Second, the

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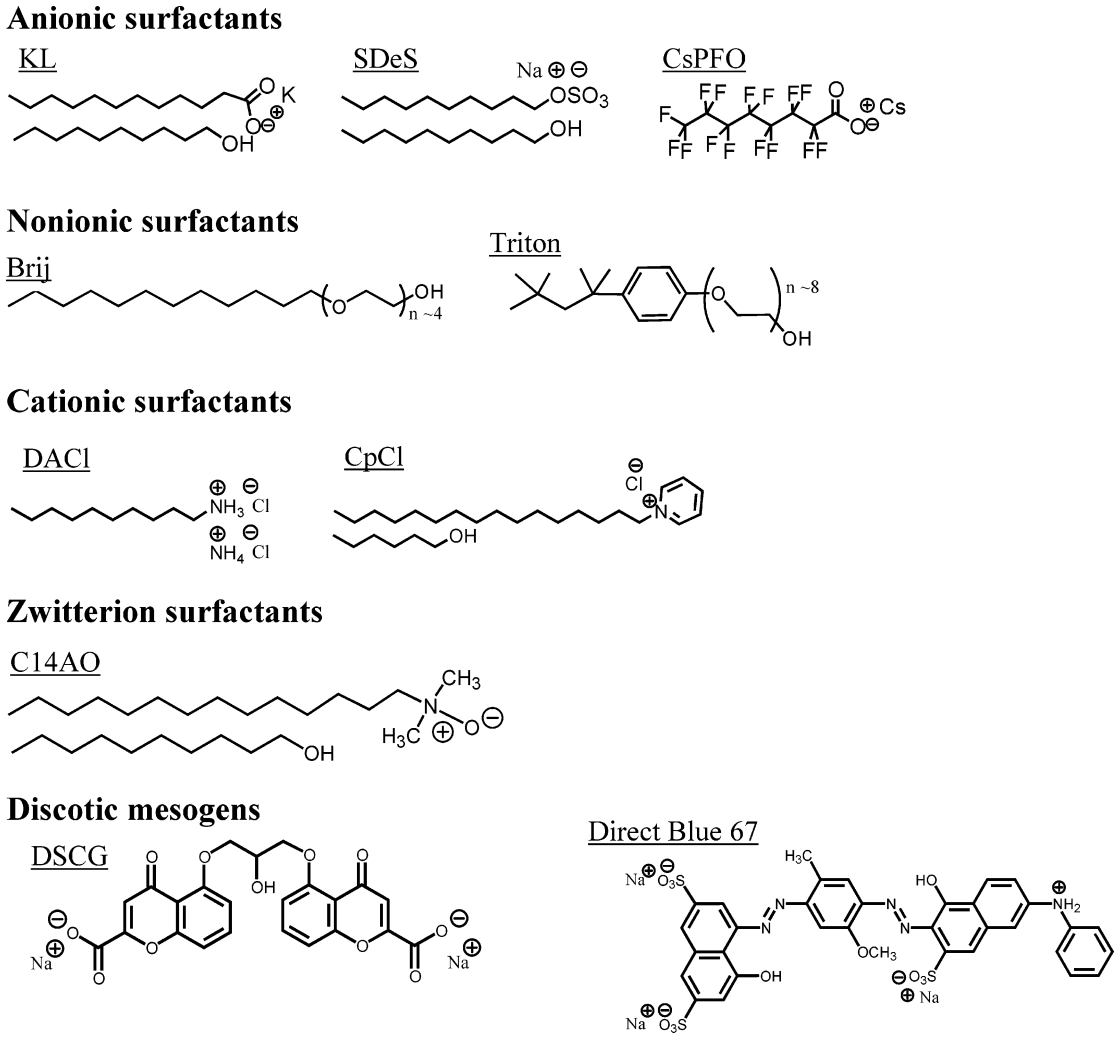

Figure 1. Structures of molecules used in the study reported in this paper that form lyotropic LCs. See text for details.

mesogens should not associate with the binding domains of the proteins in a manner that prevents the receptor−ligand interaction. Third, the viscosity of the lyotropic liquid crystal should not be so high as to prevent facile diffusion of the proteins into the vicinity of each other so as to permit binding interactions. Our study surveyed five classes of lyotropic liquid crystals that incorporated a broad range of chemical functionality and molecular architectures in an attempt to identify lyotropic LCs that fulfilled the above-listed criteria. We focused our study on antibody−antigen binding where the antibody is dissolved in the lyotropic liquid crystal and the antigens are immobilized on surfaces.

The five classes of lyotropic liquid crystals described in this paper are based on anionic, nonionic, cationic, and zwitterionic surfactants and chromonic lyotropes (Figure 1). These lyotropes comprise both amphiphilic molecules, such as anionic, cationic, and nonionic surfactants, as well as nonamphiphilic mesogens, the so-called chromonic lyotropes.[14] The amphiphiles studied form a broad spectrum of microstructures and mesophases including micelles, vesicles, nematic, columnar (hexagonal), lamellar, and cubic phases. Among the different mesophases, the lyotropic nematic phase (Figure 2) emerged from our study as one that is particularly promising as a biocompatible liquid crystalline phase. The nematic phases comprise nonspherical micelles, either columns or disks, that give rise to either cylindrical nematic (N_C) phases[15,16] or disk nematic (N_D) phases, respectively.[17,18] Because the concentration of amphiphiles in these phases is low, and because the mesophase does not possess the long-range positional order found in other mesophases (e.g., hexagonal phases), the viscosity of the nematic phase is low and thus permits rapid transport of proteins.

Lyotropic liquid crystals formed from surfactants have been widely described in the literature, and we summarize in Table 1 the compositions and properties of the LCs formed from surfactants that were used in the study reported in this paper. We also note that many past studies have investigated the interactions of surfactants and proteins. While some anionic surfactants such as sodium dodecyl sulfate are known to denature protein,[19] nonionic surfactants such as Triton X114 are often used to stabilize protein structure and reduce

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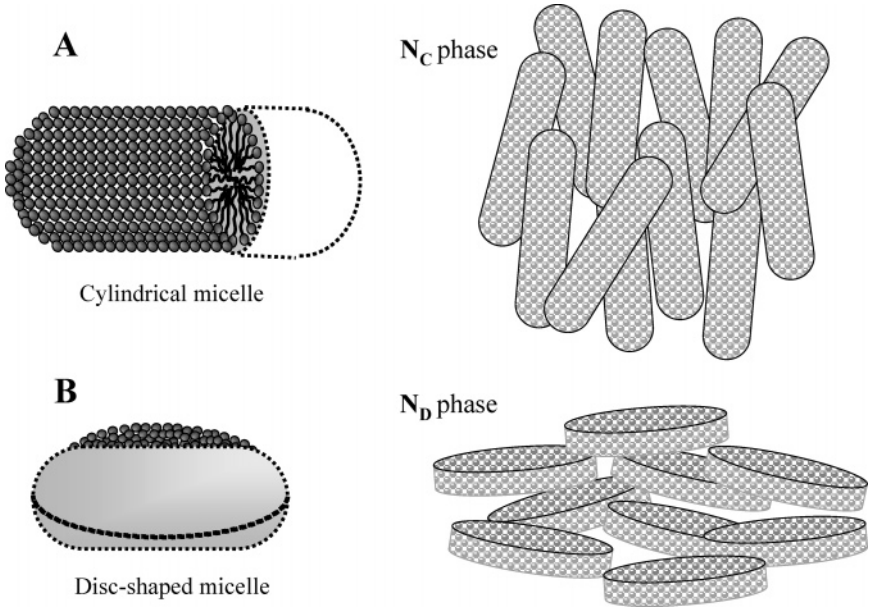

Figure 2. Schematic illustrations of nematic lyotropic LCs comprised of nonspherical micelles. (A) Cylindrical micelles give rise to a cylindrical (N_C) nematic phase. (B) Disk-shaped micelles give rise to a disklike (N_D) nematic phase.

Table 1. Composition and Properties of Lyotropic LCs and the Binding Ability of Antihuman IgG in Lyotropic LCs at 23 °C

	components	wt %	phase	observed birefringen ce	observed viscosity[a]	antibody binding
anionic surfactants	SDeS/C_{10}OH/H_2O[b]	34.7/7.0/ 58.3	N_C[c]	low	low	−
	CsPFO/H_2O[d]	58/42	N_D[e]	low	medium	−
	KL/C_{10}OH/H_2O[f]	29.4/6.6/ 64.0	N[g]	medium	low	−
nonionic surfactants	Brij 30/H_2O[h]	8/92	micelles	none	low	+
		50/50	meso[i]	low	high	−
	Emulgene/H_2O	7/93	unknown	none	low	+
	Triton 114/H_2O[h]	8/92	isotropic	none	low	+
		60/40	lamellar	low	high	−
cationic surfactants	DACl*/H_2O[j]	39/61	N_D	low	low	−
	CPCl/C_6OH/brine[k]	35/21/44	N	low	medium	+
zwitterionic surfactants	C14AO/C_{10}OH/H_2O[l]	20/3/77	isotropic	none	low	+
		23/3/74	N	medium	high	+
chromonic mesogens	DSCG/H_2O[m]	8/92	isotropic	none	low	+
		11/89	N	high	low	+
	Direct Blue 67/ Emulgene/H_2O[n]	10/7/83	N	low	low	−

[a] Viscosity was evaluated by observation of the flow of the lyotropic liquid crystal when the LC was tilted against gravity; low: flow; high: does not flow. [b] References 15 and 34. [c] N_C: cylindrical nematic phase. [d] References 31−33 and 35−37. [e] N_D: disk nematic phase. [f] Reference 38. [g] N: lyotropic nematic phase, detail morphology unknown. [h] See the section on "Preparation of Lyotropic LCs". [i] Meso: unknown birefringent phase. [j] References 41 and 42; DACl* is a mixture of DACl and NH_4Cl (20:1 by weight). [k] Reference 43; Brine: 0.2 M NaCl. [l] Reference 44. [m] References 27 and 30. [n] References 31−33.

nonspecific adsorption on surfaces.[20] Furthermore, complex aggregates and gel phases are known to form when some proteins are mixed with surfactants with charged headgroups.[21,22] In contrast to surfactant-based mesophases, lyotropic LCs phases formed from nonamphiphilic molecules have been less widely described. The nonamphiphilic mesogens typically have a rigid aromatic core and hydrophilic functional groups on the periphery of the aromatic core.[23−26] Because this class of lyotropic liquid crystal consists of lyotropes that are neither amphiphilic, nor entirely discotic, they were named chromonic by Attwood and co-workers.[14,23] The exact morphology and molecular organization of chromonic lyotropic liquid crystals has not been established, although it is clear that their mesophases share properties found in nematic phases and phases containing lamellae.[27−29]

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We note that the mesophases formed by chromonic liquid crystals arise from a set of molecular interactions that are fundamentally different from those leading to the formation of mesophases formed by amphiphilic molecules. While the mesophases formed by amphiphilic molecules are largely due to the hydrophobic interactions of the aliphatic chains, the formation of chromonic liquid crystals are thought to be due to the stacking of aromatic rings and hydrogen bonding of the functional groups that decorate the periphery of the chromonic mesogens as well as the hydrophobic interactions between the aromatic rings.[14] In our study, we included two chromonic liquid crystals, one based on disodium cromoglycate (DSCG)[30] and the other based on a mixture of a dye molecule called Direct Blue 67 and a nonionic surfactant (Emulgene).[31−33] Both of these chromonic liquid crystals are reported to exhibit nematic phases.

Experimental Section

Materials. The glass microscope slides were Fisher's Finest, premium grade obtained from Fisher Scientific (Pittsburgh, PA). Direct Blue 67 was obtained from Orichem International Ltd (Hangzhou, P. R. China). Emulgene 108 was kindly provided as a gift by Kao Specialties Americas LLC (Mahwah, NJ). HeLa (human epitheloid cervical carcinoma) cells (#CCL-2) and vesicular stomatitis virus (VSV) Indiana strain (VR-1238) were purchased from ATCC (Manassas, VA). Polyclonal FITC-conjugated anti-VSV antibody (#670-VDV) was obtained from National Veterinary Services Labs (Ames, Iowa). All other chemicals were purchased from Sigma-Aldrich Chemicals (Milwaukee, WI) and used as received. All aqueous solutions were prepared with deionized water having a resistivity greater than18.2 MΩ cm (Milli-Q^{plus}, Millipore, Bedford, MA).

Preparation of Lyotropic Liquid Crystals. *Anionic Surfactants*. A lyotropic LC containing sodium decyl sulfate (SDeS) was prepared as a ternary system by mixing 34.7 wt % of SDeS, 7.0 wt % of decanol, and 58.3 wt % of water.[15,34] A lyotropic LC containing cesium pentadecafluorooctanoate (CsPFO) was prepared as a binary system by mixing 58 wt % of CsPFO and 42 wt % of water.[31−33,35−37] Cesium pentadecafluorooctanoate was prepared by mixing equal molar amounts of cesium hydroxide and pentadecafluorooctanoic acid at 0 °C in methanol. The methanolic solution was concentrated in vacuo and the CsPFO recovered by recrystallization from methanol. A lyotropic LC containing potassium laurate (KL) was prepared as a ternary system by mixing 29.4 wt % of KL, 6.6 wt % of decanol, and 64.0 wt % of water.[38] Potassium laurate was prepared by combining equal molar amounts of potassium hydroxide and lauric acid in methanol. The solids were concentrated in vacuo, warmed, and recrystallized from methanol. The crystalline potassium laurate was mixed directly with decanol and water in the above-indicated proportions.

Nonionic Surfactants. A lyotropic LC containing Brij 30 was prepared by mixing Brij 30 and water at different weight ratios until phases were obtained that exhibited birefringence using a polarizing microscope with 4× magnifications. Based on this procedure, we investigated two compositions for their compatibility with antibody−antigen binding: (i) 8 wt % of Brij 30 and 92 wt % of water, which corresponds to an isotropic solution of micelles, and (ii) 50 wt % of Brij 30 and 50 wt % of water, which affords a viscous mesophase with detectable birefringence. We prepared two binary mixtures of Triton X114 and water. One mixture comprised of 8 wt % of Triton X114 and 92 wt % of water, which corresponded to an isotropic solution. The other composition comprised 60 wt % of Triton X114 and 40 wt % of water, which corresponds to a viscous mesophase that is reported to be lamellar.[39,40] We also prepared an Emuglene surfactant solution containing 7 wt % of Emuglene and 93 wt % of water.

Cationic Surfactants. A lyotropic LC containing decylammonium chloride (DACl) was prepared as a ternary system by mixing 39 wt % of 1:20 NH_4Cl:DACl by weight and 61 wt % of water.[41,42] Lyotropic LCs containing cetylpyridinium chloride (CPCl) were prepared as quasi-ternary systems by mixing 35 wt % of CPCl, 44 wt % of an aqueous solution containing 0.2 M NaCl, and 21 wt % of hexanol.[43]

Zwitterion Surfactants. We prepared two lyotropic LCs containing tetradecyldimethyl-amineoxide (C14AO). First, we mixed 23 wt % of C14AO, 3 wt % decanol, and 74 wt % water.[44] The solution was agitated on a Lab-Line Rotator (Fisher Scientific Inc., Pittsburgh, PA) overnight to make a homogeneous solution and then centrifuged to reduce the foaming. A second lyotropic LC was prepared containing 20 wt % of C14AO, 3 wt % decanol, and 77 wt % water.

Chromonic Mesogens. Lyotropic LCs containing disodium cromoglycate (DSCG) were prepared by mixing 11 wt % of DSCG and 89 wt % of water.[30] The solution was equilibrated for 12 h prior to use. A second lyotropic LC was prepared containing 8 wt % of DSCG and 92 wt % of water. Both of the lyotropic LCs afforded nematic phases. Whereas the solution containing 11 wt % of DSCG forms a nematic mesophase below ca. 25 °C, the solution containing 8 wt % of DSCG is nematic below ca. 15 °C.[27,30] We also prepared lyotropic LCs containing the dye molecule Direct Blue 67. This LC was prepared as a ternary system containing 10 wt % of Direct Blue 67, 7 wt % of Emulgene, and 83 wt % of water. This composition affords a nematic phase.[31−33]

Covalent Modification of Glass Surfaces with Isocyanate Groups. The glass substrates were functionalized with isocyanate as described in a previous report by Stoddart and references therein (Figure 3).[45] In brief, the glass slides were incubated in a toluene solution containing 3% w/v of 3-(triethoxysilyl) propyl-isocyanate at 40 °C for 8 h. The glass was then rinsed with toluene, hexane, and ether and dried thoroughly with a stream of nitrogen.

Preparation of Lyotropic Liquid Crystals Containing Antibodies. Antibody (either FITC-conjugated antihuman IgG or FITC-conjugated antibiotin IgG) was first dissolved in PBS (pH7.4) to afford aliquots containing 4 μM of antibody. Ten microliters of the solution containing 4 μM antibody in PBS was added directly

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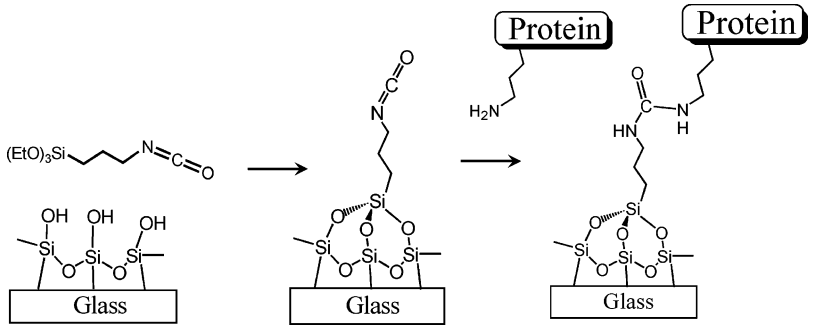

Figure 3. Schematic illustration of procedure used to immobilize antibodies on glass using isocyanate-based chemistry.

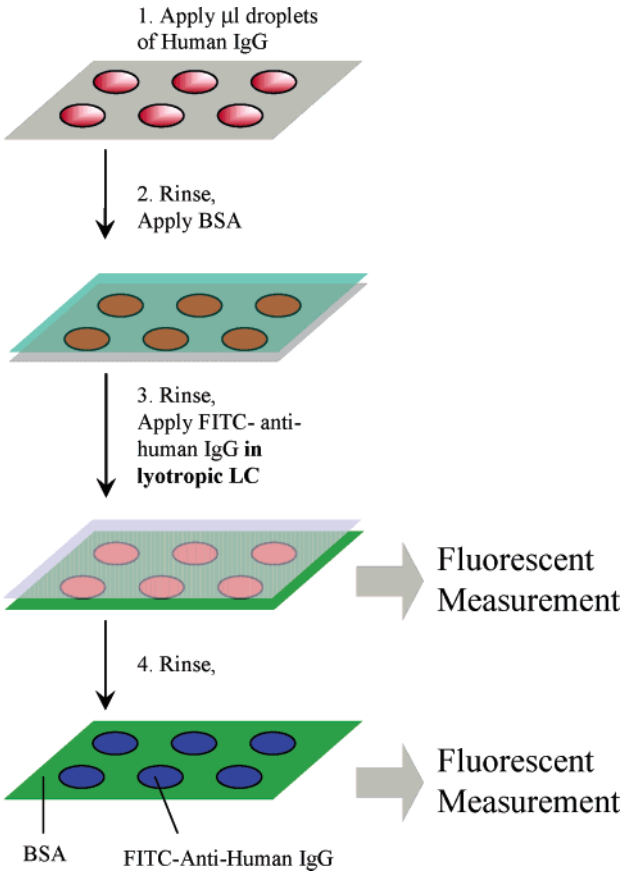

Figure 4. Experimental procedure used to immobilize human IgG and BSA on isocyanate-derivatized glass to create circular domains of human IgG surrounded by BSA and to subsequently measure binding of antihuman IgG to the immobilized human IgG.

to 190 μL of the desired lyotropic LC to give a final concentration of 200 nM of antibody in the lyotropic LC.

Antibody-Immobilized Antigen Recognition in Lyotropic Liquid Crystals. First, we spotted multiple droplets of a solution containing human IgG onto the isocyanate-derivatized glass (Figure 4). The glass was then incubated for 3 h in a covered Petri dish containing wet cotton balls to prevent evaporation of the droplets. After the glass side was sequentially rinsed with phosphate-buffered saline (PBS, pH 7.4) and deionized water and dried under a stream of N_2(g), the surface was blocked by incubation of drops of aqueous solution containing bovine serum albumin (BSA) in PBS (pH 7.4, 1 mg/ mL) on the surface for 1 h. The BSA treated arrays of human IgG were then rinsed with PBS (pH 7.4) and deionized water and dried with a stream of N_2(g). Lyotropic LC solution containing 200 nM of FITC-conjugated antibody (either FITC-conjugated antihuman IgG or FITC-conjugated antibiotin IgG) was applied over the whole surface of the human IgG-BSA-treated glass slides for 4 h. The glass slide was then rinsed with PBS (pH 7.4) and deionized water. The antibody bound to the surface was imaged by using fluorescence microscopy.

In a second set of experiments, drops of lyotropic LCs containing 200 nM of antibody were placed directly on the glass surface presenting human IgG blocked with BSA. A cover slip (0.17 mm in thickness) with two strips of double-sided tape was placed on top of the droplet of the lyotropic LC. Fluorescence microscopy was then used to image the binding of the labeled antibodies to the human IgG at intervals of 5 min.

Antibody Binding to Vesicular Stomatitis Virus (VSV) in HeLa Cells Immersed in Lyotropic Liquid Crystals. HeLa (human epitheloid cervical carcinoma) cells were grown on cover slips in a 12-well tissue culture plate until the cells reached a confluency of 95% before inoculation with VSV Indiana strain (VR-1238, American Type Culture Collection (ATCC), Manassas, VA) at a multiplicity of infection of 0.01 (i.e., using a proportion of one infectious virus particle per 100 cells). Viruses were allowed to replicate for 1 day at 37 °C to express viral antigens on the surfaces of the cells. The cell culture medium was removed and the cells were fixed with a solution mixture of 70% methanol/30% acetone. The solvent was removed after 5 min at room temperature and cells were rinsed in PBS.

Using stock antibody protein concentrations for FITC-conjugated anti-VSV antibody and FITC-conjugated antibiotin IgG of 5.17 and 1.80 mg/mL, respectively, the FITC-conjugated anti-VSV antibody and FITC-conjugated antibiotin IgG were added into PBS or 15% DSCG solution with the dilution of 1:20 and 1:7, respectively, to achieve antibody concentrations of 0.26 mg/mL. The antibodies were then incubated on the cells at room temperature for 1 h in the dark and the cells washed with PBS 4 times before mounting the cover slip with Mowiol solution on a glass slide.[46] The fluorescence was examined in cells with UV illumination using an Olympus IX70 inverted microscope.

Results and Discussion

Influence of Lyotropic Liquid Crystal on Binding of Antibody to Surface-Bound Antigen. Our first experiments sought to determine if we could image the binding of FITC-labeled antihuman IgG to human IgG covalently immobilized on the surfaces of glass microscope slides when the FITC-labeled antihuman IgG was dissolved in a lyotropic LC. We note here that the human IgG was covalently attached to the surface of the glass slide because the high surfactant content of a number of the lyotropic mesophases resulted in the removal of proteins physically adsorbed to surfaces. We performed our first experiments using 200 nM FITC-labeled

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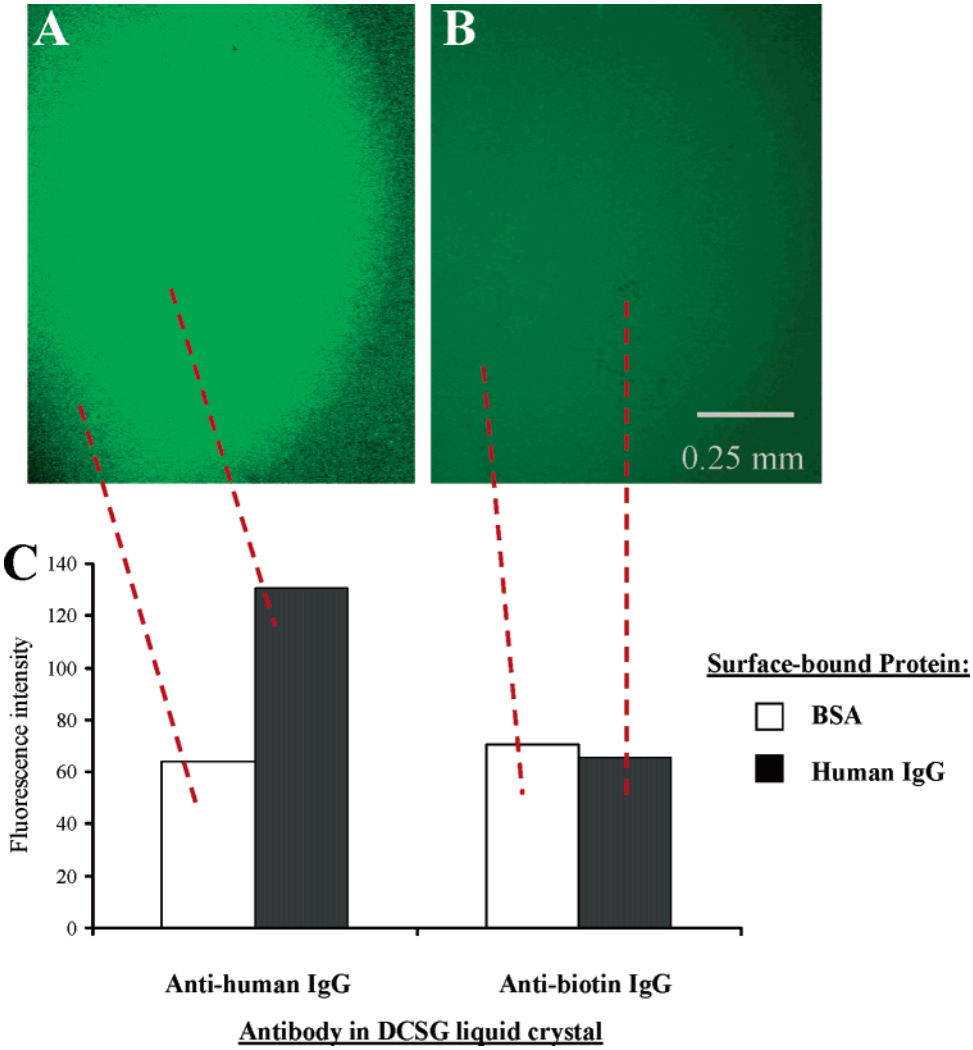

Figure 5. (A) Fluorescent images of a film (15 μm in thickness) of aqueous DSCG (8 wt %) containing 200 nM FITC-conjugated antihuman IgG in contact with a surface patterned with circular domains of human IgG surrounded by BSA. (B) Same as (A) but using 200 nM of FITC-conjugated antibiotin IgG (nonspecific antibody). The images were taken 7 min after the lyotropic LCs containing antibodies were contacted with the patterned surfaces. (C) Intensity of fluorescence calculated from (A) and (B).

antihuman IgG dissolved in an aqueous solution containing 8% DSCG at room temperature. At room temperature, as noted in the Materials and Methods section, an aqueous solution containing 8% DSCG is not liquid crystalline. In this experiment, we sought to determine if the chemical functionality of the DSCG molecules prevented specific binding of the antibody to the surface-immobilized antigen. The experiment was performed by placing a drop of the solution containing DSCG and antibody onto the surface covered with antigen and then covering the drop with a second glass slide spaced from the surface-immobilized antigen by a distance of 15 μm. Figure 5 shows fluorescent micrographs obtained approximately 15 min after contact of the solution containing antibody with the surface. Inspection of Figure 5A reveals the presence of a diffuse circular domain of fluorescence intensity that is higher than the fluorescence intensity outside the circle (see below for quantitation). These domains appeared in the fluorescence micrograph over a period of approximately 7 min following contact of the solution with the surface. Because a similar experiment (Figure 5B) performed with FITC-conjugated antibiotin IgG (nonspecific control IgG) did not lead to the formation of the bright circular domains, we conclude that the bright domain is the result of the recruitment and binding of FITC-labeled antihuman to the surface-immobilized antigen. We note that the fluorescence intensity seen in Figure 5 contains contributions from FITC-labeled molecules that are captured on the surface as well as those molecules dissolved in the bulk of the solution. We interpret the enhanced fluorescence intensity in the circular domains to result from binding of FITC-conjugated antihuman IgG in the circular regions of the surface-bound human IgG. The binding localized the FITC-conjugated antihuman IgG in the focal plane of the microscope, leading to enhanced brightness in this region. We note that, over the duration of the experiment (15 min), the concentration of FITC-conjugated antihuman IgG in the bulk solution is likely lower in the region of the solution above the surface-bound human IgG than in the region of the solution above the surface-bound BSA. This situation appears likely because we calculate the time required for lateral diffusion of antibody across the circular domain of immobilized human IgG to be longer than the duration of the experiment. We estimate the diffusion time as $t = d^2/D$, where d is the diameter of the circular region of surface-

bound human IgG (~1 mm), D is the effective diffusion coefficient (~5×10^{-7} cm^2 s^{-1}). This expression leads to an estimate of 6.6 h.[47,48]

We quantified the selectivity of binding of antihuman IgG for surface-immobilized human IgG by comparing the intensity of the fluorescence measured using FITC-labeled antihuman IgG to that measured using FITC-labeled antibiotin IgG (Figure 5C). Using FITC-conjugated antihuman IgG that was dissolved in 8% DSCG, we measured the ratio of the fluorescence on the regions of the surface with human IgG to be twice that of the fluorescence intensity measured on the regions blocked with BSA. In contrast, there was no measurable difference in the fluorescence of the same two regions of a surface when FITC-conjugated antibiotin IgG was incubated on the surface. These results are consistent with the retention of high levels of binding specificity of antibodies in aqueous solutions containing DSCG.

We confirmed the above conclusions regarding the origin of the fluorescence intensity in Figure 5A by removing the aqueous solution containing DSCG and nonbound antibody from the surface by rinsing the surface with PBS and water. Inspection of Figure 6 shows that following a 4 h of incubation of FITC-conjugated antihuman IgG on the surface, regions of the surface presenting human IgG are associated with high intensities of fluorescence whereas the surrounding regions presenting BSA do not give rise to a significant fluorescence signal. Streaks of fluorescence are apparent on one side of each circular domain of human IgG. These streaks appear on the same side of each circular domain and are caused by the deposition of human IgG on these regions of the surface during the rinsing of the droplets containing human IgG from the surface (during the preparation of the surface, step 2 in Figure 4). In contrast to Figure 6A, when the glass slides were treated with the control antibody (FITC-conjugated antibiotin IgG) dissolved in the aqueous solution of DSCG, no fluorescent signal was detected over the entire glass slide (Figure 6B). Furthermore, we applied solutions containing either FITC-conjugated antihuman IgG or antibiotin IgG at opposite ends of a surface patterned with circular domains of human IgG surrounded by BSA and allowed the two solutions to mix by lateral diffusion. We were able to identify a diffusion front corresponding to a transition from circular domains with fluorescent signal and to regions of the surface with no fluorescent signal. The diffusion front passing through a single circular domain of surface-bound IgG is shown in Figure 6C. In summary, the results described above confirm the specificity of binding of the antibodies to the surface-bound antigen in the presence of 8% DSCG. They further indicate that the binding is operationally irreversible under the conditions of the experiments described above.

Next, we examined the binding of FITC-conjugated antihuman antibody dissolved in a nematic phase containing DSCG. We increased the concentration of DSCG to 11 wt % in water because this concentration affords a nematic phase

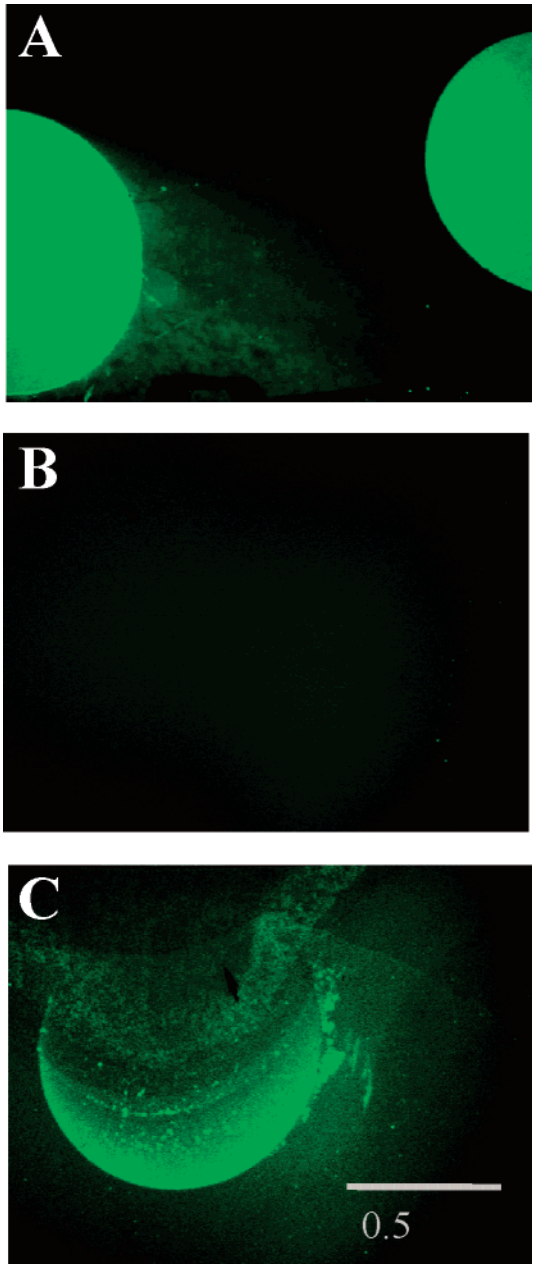

Figure 6. (A) Fluorescent images of a surface patterned with circular domains of human IgG after a film (15 μm in thickness) of aqueous DSCG (8 wt %) containing 200 nM FITC-conjugated antihuman IgG was incubated on the surface and then rinsed using PBS and water. (B) Same as (A) but using 200 nM of FITC-conjugated antibiotin IgG (nonspecific antibody). (C) Fluorescent image obtained when drops of aqueous DSCG containing antihuman IgG and antibiotin IgG were fused on the surface patterned with circular domains of human IgG. See text for details.

of DSCG at the ambient temperature (22 °C) of our laboratory. We found that the binding activity of FITC-conjugated antibody to be indistinguishable from that described above using 8% DSCG.

Surveying the Binding Ability of Antihuman IgG in Lyotropic Liquid Crystals. Using the procedures described above, next we surveyed the binding ability of antihuman IgG to surface-immobilized human IgG in the lyotropic LCs listed in Table 1. Table 1 summarizes the composition of the LCs, the types of mesophases, their optical properties and viscosity, and whether we measured specific binding of antihuman IgG to surface-immobilized human IgG. Inspec-

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tion of Table 1 reveals that mesophases formed from anionic surfactants (sodium decyl sulfate (SDeS), cesium pentadecafluorooctanoate (CsPFO), and potassium laurate (KL)) failed to permit specific binding of antihuman IgG to the surface-immobilized human IgG. Past studies have established that sodium dodecyl sulfate is effective at denaturing proteins.[19] A likely explanation for the observations reported above is that the anionic surfactants used to form the mesophases have denatured the antibodies. It is also interesting to note that we have previously reported the use of LCs formed from KL to detect the presence of antibiotin IgG bound to surfaces presenting biotin. The presence of the bound antibody on the surface was reported as a disordering of the LC contacted with the surface.[38] This result indicates that while lyotropic LCs may prevent the antibodies from binding to surface-bound antigens, the presence of the LC does not necessarily disrupt antigen−antibody complexes already formed on surfaces.

Inspection of Table 1 also reveals that the lyotropic LCs formed from the two nonionic surfactants used in our study (either 50 wt % Brij 30 or 60 wt % Triton X114) prevented the binding of antihuman IgG to surface-bound human IgG. Because Brij 30 and Triton X114 are widely used in protein binding assays to limit nonspecific adsorption of proteins at hydrophobic surfaces, we speculated that the absence of bound antihuman IgG was not caused by denaturation of the proteins but rather slow mass transport in the highly viscous phases formed by Brij 30 and Triton X114 at the concentrations stated above. To test this proposition, we performed experiments to determine if specific binding could be detected from lower viscosity aqueous solutions containing lower concentrations of the nonionic surfactants. When solutions containing 8 wt % of either Brij 30 or Triton X114 were used, high levels of specific binding of antihuman IgG to surface-immobilized human IgG were measured by using fluorescence microscopy. Because the antibody−antigen binding is not prevented in the aqueous solutions containing low concentration (8 wt %) of the two nonionic surfactants, the absence of measurable binding at high concentration of nonionic surfactants is consistent with slow diffusion of antibodies within these phases. We cannot, however, also rule out the possibility that the high concentrations of surfactants induced aggregation of the antibodies, which might, for example, prevent their recognition of antigens.

In contrast to the results described above for the nonionic and anionic surfactants, we observed mesophases formed from some cationic surfactants to prevent specific binding of antihuman IgG to surface-immobilized human IgG (DACl) whereas others did permit binding events to occur (CPCl). Both DACl and CPCl form nematic phases under the conditions used in our study. Similar to the conclusions extracted from our results with nonionic and anionic surfactants, the result with the cationic surfactants supports our view that the chemical functionality of the surfactant plays a dominant role in determining whether binding activity of the antibodies is observed in the LC. Although the mechanisms by which the nature of the polar headgroups of surfactants influence the interactions of the surfactants and proteins is not well understood, surfactants with zwitterionic headgroups are generally observed to be less denaturing than surfactants with anionic or cationic headgroups.[49,50] Our observations based on lyotropic LCs formed from the zwitterionic surfactants tetradecyldimethyl-amineoxide (C14AO) are consistent with the results of past studies of protein−surfactant interactions. We observed antibody binding in both isotropic solutions (20 wt % of C14AO at ca. 23 °C) and mesophases (23 wt % of C14AO at ca. 23 °C) formed by C14AO. We note here that although many amphiphilic surfactants are known to denature proteins, the correlation between the chemical structure and extent of protein denaturation is still under investigation.[51] For simple ions, there do exist empirical rankings of the tendency of ions to stabilize or destabilize proteins (such as the Hofmeister series). However, the mechanism by which ions stabilize or destabilize protein structure is not well understood.[51−53]

We also investigated the binding ability of antibodies dissolved in lyotropic mesophases formed from nonamphiphilic molecules such as DSCG (also see above) and Direct Blue 67. The aggregates present in these phases arise not from hydrophobic/hydrophilic interactions of the aliphatic chains and polar headgroups of the mesogens but rather by the stacking of the aromatic rings and through interactions of the dipole and hydrogen bonding groups in the periphery of the aromatic rings. As noted above, in mesophases formed by DSCG, we did observe the specific binding of antihuman IgG to surface-bound human IgG (Table 1). In contrast to the result obtained with the mesophase of DSCG, however, no binding of antihuman IgG was detected when a mesophase was formed from the mixture of Direct Blue 67 and Emulgene. Because our results indicate that Emulgene alone supports antibody−antigen binding (see Table 1), we conclude that the lack of antibody−antigen binding is due to Direct Blue 67. Because the viscosity of the Direct Blue 67/Emulgene lyotropic LC is low, the lack of binding of antihuman IgG in lyotropic LCs containing Direct Blue 67 may be due to denaturation caused by the Direct Blue 67. Although both chromonic mesogens are nonamphiphilic, it is interesting that DCSG permits antibody−antigen binding whereas Direct Blue 67 prevents the antibody−antigen binding. We note here that past studies have established that a range of nonamphiphilic molecules such as guanidinium ions can denature proteins. Thus, amphiphilicity is not required for a molecule to function as a protein denaturant. Structural requirements for molecules to function as denaturants have not been defined.

The results above, when combined, reveal that three lyotropic LCs (CPCl, C14AO, and DSCG) permit the binding of antihuman IgG and surface-bound human IgG. Among these three lyotropic LCs, DSCG appears to be the most useful one for two reasons. First, at relatively low concentration, the DSCG lyotropic LC exhibits high birefringence but low viscosity. Second, because DSCG is not amphiphilic, this lyotrope and its structural variants may not damage the

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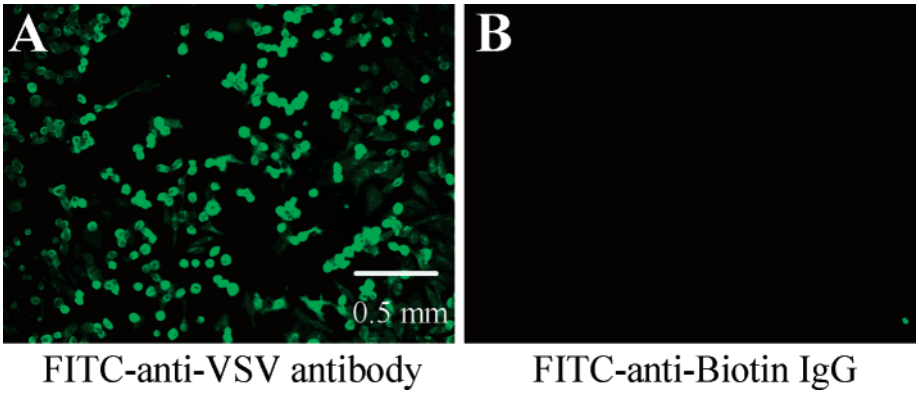

Figure 7. Fluorescence images obtained after incubation of (A) FITC-conjugated anti-VSV dissolved in a LC containing 15 wt % of DSCG with HeLa cells inoculated with VSV. (B) FITC-conjugated antibiotin IgG. See text for details.

membrane structure and thus be compatible with enveloped structures such as viruses, bacteria, and mammalian cells.

Antibody Binding to Vesicular Stomatitis Virus (VSV) in HeLa Cells Immersed in Lyotropic Liquid Crystals. We next sought to determine if it was possible to measure specific binding between an antibody directed to a viral antigen where the viral antigen is presented at the surface of a mammalian cell immersed in a LC phase prepared from DSCG. As described in the methods section, we inoculated cells with vesicular stomatitis virus (VSV),[10] fixed the cells, and then incubated the cells in a mesophase formed from 15 wt % of DSCG at room temperature. We used a polyclonal FITC-conjugated anti-VSV antibody that is specific for envelope proteins of VSV and FITC-conjugated antibiotin IgG as a control. The concentration of each antibody in the LC prepared from DSCG was ca. 0.26 mg/mL, and the antibodies were incubated on the fixed cells for 1 h. Figure 7 shows the FITC fluorescence of antibody-bound VSV (inoculated in HeLa cells) treated with the two antibodies. Whereas fluorescence was detected when antibody specific for VSV (FITC-conjugated anti-VSV antibody) was used, no fluorescence was detected when FITC-conjugated antibiotin IgG was used. These results support two inferences. First, the binding of anti-VSV antibody in DSCG to surface-bound VSV is highly specific. Second, lyotropic LCs formed from DSCG may provide the basis of a versatile birefringent solvent that is compatible with a wide range of antibodies (not just antihuman IgG).

Conclusions

In conclusion, we have screened 10 lyotropic LCs for their possible use as birefringent solvents that permit antibodies to recognize surface-bound antigens (Table 1). The results of this survey suggest that the molecular structure of the lyotrope plays a primary role in determining whether the lyotropic LC will permit binding of antibodies to surface-bound antigens. While denaturation of the antibody appears likely to be the main cause of loss of antibody binding ability, other properties of the LC phases such as high viscosity and/or surfactant-induced protein aggregation may also prevent binding of antibodies to surface-bound antigens. Our study has revealed three lyotropic LCs, cetylpyridinium chloride (CPCl), tetradecyldimethylamineoxide (C14AO), and disodium cromoglycate (DSCG) to permit the specific binding of antibody to surface-bound antigens. Among these three lyotropic LCs, DSCG is particularly promising because of its high birefringence and low viscosity. We also demonstrate specific binding of antibodies to virus-infected cells using antibodies to the virus that were dissolved in LCs prepared from DSCG. When combined, these results identify materials and principles that will guide fundamental studies of biomolecular interactions in LCs as well as enable the development of new classes of biological assays performed using LCs.

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