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Compatibility of lyotropic liquid crystals with viruses and mammalian cells that support the replication of viruses

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Abstract

We report a study that investigates the biocompatibility of materials that form lyotropic liquid crystals (LCs) with viruses and mammalian cells that support the replication of viruses. This study is focused on aqueous solutions of tetradecyldimethylamineoxide ($C_{14}AO$) and decanol (D), or disodium cromoglycate (DSCG; $C_{23}H_{14}O_{11}Na_2$), which can form optically birefringent, liquid crystalline phases. The influence of these materials on the ability of vesicular stomatitis virus (VSV) to infect human epitheloid cervical carcinoma (HeLa) cells was examined by two approaches. First, VSV was dispersed in aqueous $C_{14}AO + D$ or DSCG, and then HeLa cells were inoculated by contacting the cells with the aqueous $C_{14}AO + D$ or DSCG containing VSV. The infectivity of VSV to the HeLa cells was subsequently determined. Second, VSV was incubated in LC phases of either $C_{14}AO + D$ or DSCG for 4 h, and the concentration (titer) of infectious virus in the LC was determined by dilution into cell culture medium and subsequent inoculation of HeLa cells. Using these approaches, we found that the LC containing $C_{14}AO + D$ caused inactivation of virus as well as cell death. In contrast, we determined that VSV retained its infectivity in the presence of aqueous DSCG, and that greater than 74–82% of the HeLa cells survived contact with aqueous DSCG (depending on concentration of DSCG). Because VSV maintained its function (and we infer structure) in LCs formed from DSCG, we further explored the influence of the virus on the ordering of the LC. Whereas the LC formed from DSCG was uniformly aligned on surfaces prepared from self-assembled monolayers (SAMs) of $HS(CH_2)_{11}(OCH_2CH_2)_4OH$ on obliquely deposited films of gold in the absence of VSV, the introduction of 10^7–10^8 infectious virus particles per milliliter caused the LC to assume a non-uniform orientation and a colorful appearance that was readily distinguished from the uniformly aligned LCs. Control experiments using cell lysates with equivalent protein concentrations but no virus did not perturb the uniform alignment of the LC.

Keywords: Compatibility; Liquid crystals; Virus; Cells

1. Introduction

Liquid crystals (LCs) represent a remarkable state of matter because molecules within LCs have translational mobilities similar to those of isotropic liquids yet they show orientational and sometimes positional ordering similar to that of solid crystals [1]. Although it has long been known that biological molecules form lyotropic LCs (solvent containing LCs; in our study, the solvent is water), it is only recently that synthetic LCs have received substantial attention because of their potential applications in biological studies, including reporting of the presence of viruses captured at surfaces [2],

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amplification of receptor–ligand binding involving proteins at surfaces [3–7], reporting of enzymatic activity [8], imaging of mammalian cells [9,10], and measurement of the presence of environmental agents such as organophosphonates [11]. In these past studies, the interactions between the LCs and target species changed the ordering of the LCs in a manner that was readily imaged by using polarized light. Whereas a past study [2] has reported that viruses captured at surfaces can be detected by observation of the orientational behavior of LCs near these surfaces, the influence of the LC on the ability of the virus to perform functions such as infect a cell and replicate was not investigated. The goal of the study reported in this paper was to explore the biocompatibility of materials that form LCs with a virus as well as biocompatibility of such materials with cells that support the replication of the virus.

Past studies of the compatibility of LCs with viruses and mammalian cells are limited [9]. Recently, we screened thermotropic liquid crystalline phases (LCs that do not contain a solvent, and exhibit temperature-dependent properties) composed of unique sets of functional groups to determine if the chemical functionality of the LCs correlated with the cytotoxicity of the LCs [9]. We identified several chemical groups that, when incorporated in molecules forming thermotropic liquid crystalline phases, were not toxic to cells during the 4 h incubation time nor did they affect the post-treatment proliferation of the cells [9]. In particular, we identified LCs containing fluorophenyl groups that were found to cause minimal toxicity to mammalian cells after 4 h of exposure [9]. However, to date, the effect of LCs on the structure or function of a virus, particularly the infectivity of virion to cells, has not been reported.

The study reported in this paper is motivated by the proposition that the identification of LC materials compatible with viruses and their host cells will enable investigations of the potential use of LCs to report viral infectivity. For example, LCs might be used to image the infection and release of virus from the surfaces of cells before the cells are killed by the viral infection (the cytopathic effect (CPE) of the virus, which is the conventional means of determining that a cell is infected with a virus). In this paper, we report the results of an initial search for materials that form LCs and when placed into contact with cells and virus permit viral infection to occur.

The virus used in our study is vesicular stomatitis virus (VSV), a member of the *Rhabodoviridae* family. VSV is an important pathogenic virus of cattle and swine, causing fever and vesicles in the mouth and on the feet. The structure of VSV has been defined by electron microscopy with negative staining, showing that the bullet-shaped virus is approximately 180 nm (range 125–205 nm) long and 75 nm (range 45–85 nm) wide [12]. The virion is composed of an internal core (helical RNA and nucleocapsid protein) surrounded by a viral protein matrix and an external phospholipid membrane derived from the cell in which the virus grows. The membrane is studded with viral glycoprotein projections [12,13].

In addition to reporting on the biocompatibility of LCs and viruses, in this paper we describe the results of preliminary observations regarding the influence of viruses on the order of a LC that we determined to be compatible with VSV. Because the energy required to disturb the orientational order of LCs is small, subtle interactions between targeted species such as cells and viruses and LCs can lead to changes in the orientational order of the LCs that can be imaged by using polarized light microscopy.

2. Materials and methods

2.1. Virus and cells

VSV Indiana strain (VR-1238, American Type Cell Collections (ATCC), Manassas, VA, USA) was used in this study. Human epitheloid cervical carcinoma cells (HeLa cells; ATCC #CCL-2) were grown in minimum essential medium (MEM) with Earle's salts and L-glutamine supplemented with 10% fetal bovine serum (FBS), 1 × non-essential amino acid solution for MEM, and antibiotic–antimycotic solution (100 units/ml penicillin G, 0.25 μg/ml amphotericin B, and 100 μg/ml streptomycin), and were maintained at 37 °C with 5% CO_2. The medium and supplements, with the exception of FBS (Gibco BRL, Grand Island, NY, USA), were obtained from Cellgro® (Mediatech, Inc. Herndon, VA, USA). Cells cultured in flasks with a growth area of 150 cm^2 (2×10^7 cells approx. in each flask) were infected with VSV using one infectious virus particle per 100 cells. After incubation for 48 h at 37 °C, the supernatant was collected for purification of the virus.

2.2. Purification of virus and determination of titer

The supernatant from infected HeLa cells was collected and purified as previously described [2,13]. Briefly, a crude virus particle pellet was obtained after the cell debris was removed from the supernatant and centrifuged through a 30% sucrose (w/v) cushion in STE buffer (10 mM Tris–NaCl, 0.1 M NaCl, 1 mM EDTA, pH 8.0). The pellet was resuspended in STE buffer and subjected to further purification through a buoyant density gradient (20–70% of sucrose in STE buffer) and centrifuged for 18 h in a Beckman SW41 rotor at 120,000*g* at 4 °C. After fractionation of the gradient, the virus fractions were collected and later confirmed by virus titration and immunoassay with anti-VSV antibodies. The virus was then concentrated by pelleting through another 30% sucrose (w/v) cushion and resuspended in STE buffer. This solution was then stored at −80 °C in working aliquots until needed. The titer of infectious virus particles in solution was determined by means of a plaque assay, as described elsewhere [14,15]. The titer of the virus in solution was calculated as plaque forming units per milliliter (pfu/ml).