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Imaging DNA single-strand breaks generated by reactive oxygen species using a liquid crystal-based sensor

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ABSTRACT

DNA single-strand breaks (SSBs) have attracted much interest since they are highly related to carcinogenesis and ageing. Herein, we report a new liquid crystal (LC)-based sensor for the detection of DNA SSBs generated by reactive oxygen species (ROS) created from the Fenton reaction. The adsorption of single-stranded DNA (ssDNA) onto the cationic surfactant-laden aqueous/LC interface interferes with the surfactant layer, inducing a planar orientation of the LCs. However, the DNA SSBs generated by the Fenton reaction-produced ROS result in a decrease of the electrostatic interactions between the ssDNA and cationic surfactant molecules, causing rearrangement of the surfactant layer and reorientation of the LCs back to a homeotropic alignment. The changes in orientation of the LCs caused by the DNA SSBs are simply converted and observed as a shift from a bright optical image of the LCs to a dark one under a polarized light microscope. With this simple LC-based approach, the DNA SSBs could be detected more effectively and rapidly without any complex instrumentation or intricate processes. Therefore, our research provides a novel strategy for the detection of DNA damage as well as better insight into the DNA-damaging process.

Introduction

DNA damage represents a serious and continuous threat to the genome stability in mammalian cells, and has a major impact on human health risk [1]. The most common type of DNA damage, DNA single-strand breaks (SSBs), are disconnections of one strand of the double helix, accompanied by the loss of single nucleotides or damaged 5′ or 3′ DNA termini [2]. Several different kinds of SSBs occur frequently in each cell every day through the action of potent reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anions, and hydroxyl radicals, which are readily generated by the endogenous processes of living organisms (e.g., respiratory chain, inflammation, cell injury) or by various exogenous sources (e.g., ionizing radiation, carcinogens) [3–6]. If such DNA damage is not repaired rapidly, it will cause cell death, chromosomal aberrations, or chromosomal mutation [1,7,8]. Consequently, DNA damage could be suggested to play a significant role in carcinogenesis and ageing [9–11].

Over the past decades, a number of strategies have been developed to detect and quantify DNA damage, such as the polymerase chain reaction (PCR), comet assay, Halo assay, terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay, flow cytometry, and gas chromatography-mass spectrometry (GC-MS) [12–18]. Analytical methods based on PCR are commonly used for the detection of gene-specific DNA damage, especially since the amplification stops at the damaged DNA site. Although PCR-based assays are highly sensitive and reliable, they cannot recognize and quantify the specific kind of DNA damage that has occurred [12,13]. The comet assay is employed to estimate the distribution of DNA damage in the cell population, but requires tricky techniques and is a time-consuming process [14]. Both the Halo and TUNEL assays can detect DNA damage rather easily using fluorescent dyes, but they have low sensitivity and need the additional labeling process [15,16]. In addition, although the GC-MS method is useful for the detection of oxidative DNA damage, it still has the limitation of overestimating the damage [18]. Since all of these sophisticated methods are time-consuming and require complex instrumentation, the development of a simple and effective method for detecting DNA damage is highly desired.

Liquid crystals (LCs) are one of the most promising materials in the field of chemical and biological assays. The characteristics of LCs, such as their anisotropic property and long-range orientational behavior, allow for the amplification and transduction of biomolecular interactions to an optical image that can be seen with the naked eye under a polarized light microscope [19–21]. In this regard, the LC-based sensing technique is simple, rapid, and sensitive, and does not require an additional labeling process, laborious techniques, and complex

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instrumentation [22–24]. In past studies, the surfactant-laden aqueous/LC interface had attracted much interest for use in detecting various biomolecular interactions, because it can be easily modified and designed [25,26]. Hu and Jang [25] reported that enzyme activity could be detected with the LC-based sensing technique when the products of the biological enzyme reaction disrupt the organization of the surfactant membrane and induce orientational change of the LCs at the aqueous/LC interface. Moreover, Price and Schwartz [26] revealed that ssDNAs could be adsorbed at the surfactant-laden interface and result in orientational change of the LCs. On the basis of this phenomenon, they investigated the DNA hybridization-induced reorientation of LCs at the aqueous/LC interface.

In this study, we have established a new LC-based approach for the detection of ssDNAs damaged by ROS generated from the Fenton reaction. The Fenton reaction is a simple reaction of ferrous ions with H_2O_2, producing ROS that pose a great risk to biomolecules, including DNA [27]. We attempted to investigate the activity of the Fenton reaction towards ssDNAs adsorbed at the cationic surfactant-laden aqueous/LC interface, and their close correlation with the orientational transitions of LCs. To the best of our knowledge, the LC-based sensing technique has never been employed to detect DNA SSBs generated by ROS from the Fenton reaction. Thus, this study suggests a novel and simple LC-based approach for the detection of DNA damage caused by ROS and its potential use.

Material and methods

Materials

The nematic LC, 4-cyano-4′-pentylbiphenyl (5CB), was acquired from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan; C1555). Glass microscope slides were purchased from Matunami (Osaka, Japan; S-1215). Copper specimen grids (75 meshes, pitch = 340 μm, hole = 285 μm, bar = 55 μm) were obtained from Gilder Grids (Granthan, UK). Octadecylammonium bromide (OTAB), Tris-buffered saline (TBS; 0.05 M Tris, 0.138 M NaCl, and 0.0027 M KCl, pH 8.0, at 25 °C), iron (II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), and microcapillary tubes were purchased from Sigma-Aldrich (St. Louis, MO, USA). Octyltrichlorosilane (OTS) was obtained from Alfa Aesar (Ward Hill, MA, USA). H_2O_2 (30%, w/v), *n*-heptane (anhydrous), and sulfuric acid were acquired from Daejung Chemicals & Metals Co., Ltd. (Daejeon, South Korea). Methyl alcohol, ethyl alcohol, and dichloromethane were purchased from Duksan Pure Chemicals Co., Ltd. (Yongin, South Korea). The unlabeled 20-mer ssDNA oligonucleotide (5′-TCTCAAACT AGAACCGAGTC-3′) and the fluorescently labeled 20-mer ssDNA oligonucleotide (5′-/56-FAM/TCTCAAACTAGAACCGAGTC-3′) were obtained from Mbiotech, Inc. (Hanam, South Korea). Deionized water (18.2 MΩ cm^{-1}) was acquired from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

OTS treatment of the glass microscope slide

Glass microscope slides were washed with piranha solution (70% sulfuric acid and 30% H_2O_2) as described in previously published methods [28]. Then, the piranha-cleaned glass slides were immersed in 10 mM OTS solution for 30 min, following which they were rinsed with dichloromethane and dried under a stream of nitrogen gas.

Preparation of LC films on copper grids

LC films on copper grids were prepared as reported previously [29]. In brief, copper specimen grids were placed in an eight-well chamber with the bottom fixed to the OTS-treated glass slides. Next, 1 μL of 5CB was seeped into the copper grid, and the excess 5CB was eliminated using a microcapillary tube. Since the purpose of OTS treatment of the glass slide was to induce a homeotropic anchoring of LCs on the surface, defective slides that did not indicate a homeotropic orientation of the LCs were rejected.

Fabrication of LC optical cells

The cationic surfactant (OTAB)-laden aqueous/LC interface was formed by immersing the LC-filled copper grids in the OTAB solution for 1 h at room temperature. Then, 400 μL of the aqueous solution of interest was applied to the OTAB-laden aqueous/LC interface. All aqueous solutions of interest were prepared with TBS. In the preparation of DNA SSBs, 5 μL of 50 mM $FeSO_4$ and 500 μL of H_2O_2 at a certain concentration were incubated with 500 μL of ssDNA at room temperature for 1 h under shaking condition. Each assay was carried out at least ten times independently.

Polarized light microscopy

LC optical cells were examined using a Nikon polarized light microscope (ECLIPSE LV100POL), and LC images were obtained with a Nikon digital camera (DS-2Mv) attached to the microscope, using a 5× Nikon objective lens.

Fluorescence microscopy

For fluorescence microscopy, LC optical cells were examined under a Nikon fluorescence microscope (ECLIPSE 80i), and LC images were acquired with a Nikon digital camera (DS-5M) affixed to the microscope. Single-stranded oligonucleotides labeled at the 5′ end with 5 (6)-fluorescein (5 (6)-FAM) were used for the fluorescence microscopy experiments instead of unlabeled ssDNA oligonucleotides.

Results and discussion

LC-based sensing mechanism

DNA damage, especially SSBs, has been an interesting subject in molecular cell biology, since it is closely relevant to the origins of cancer and ageing [1]. The development of an effective method to detect DNA damage and the simplification of such procedure are still required, although many kinds of detection strategies have already been reported [7]. Thus, in this study, we developed an LC-based sensing system to detect DNA SSBs more simply and rapidly. Inspired by the fact that ssDNAs adsorbed at the cationic surfactant-laden aqueous/LC interface can cause changes in the arrangement of the surfactant layer and thus induce orientational transition of the LCs [26,30], we fabricated LC optical cells with a cationic surfactant-laden LC thin film on copper grids. The self-assembled cationic surfactant layer results in homeotropic anchoring of the LCs (Fig. 1(A)), but when the ssDNA is adsorbed at the surfactant-laden aqueous/LC interface, the LC orientation changes from a homeotropic to a planar alignment owing to interaction of the ssDNA with the surfactant molecules (Fig. 1(B)). The Fenton reaction occurring at the aqueous/LC interface will produce enough ROS to damage the ssDNA, and this will reduce the interaction of the ssDNA with the surfactant molecules, inducing rearrangement of the surfactant layer and transition of the LCs back to a homeotropic orientation (Fig. 1(C)). The orientational transition of LCs induced by the Fenton reaction would be observed through their optical image change from a bright to a dark one. With this LC-based sensing mechanism, we speculated that the DNA SSBs could be simply detected.

ssDNA adsorption at the aqueous/LC interface

Since the cationic surfactant molecule was proven to induce vertical alignment of LCs at the aqueous/LC interface in previous studies [26,30], we first determined the minimum OTAB surface coverage required at the aqueous/LC interface to create the homeotropic