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Research Article

# Selection of surfactants for cell lysis in chemical cytometry to study protein-DNA interactions

Protein-DNA interactions play a defining role in many cellular processes. Studying such interactions at the single-cell level is important and challenging. Here we make the first step toward achieving this goal with chemical cytometry. Chemical cytometry utilizes capillary separation for detailed chemical analyses of single cells. The cell is injected into a capillary, lysed, and its components are analyzed by CE or capillary chromatography with highly sensitive detection. In order to apply chemical cytometry to studies of protein-DNA interactions, cell lysis must not destroy protein-DNA complexes. Surfactants represent the most practical means of cell lysis inside the capillary. This work aimed at finding surfactants and lysis conditions that do not destroy protein-DNA complexes. We studied three groups of surfactants – ionic, zwitterionic, and nonionic – with respect to their ability to lyse the cell membrane without significantly influencing the stability of protein-DNA complexes. Nonequilibrium CE of equilibrium mixtures with surfactants in the equilibrium mixtures and in the run buffer was used to measure the equilibrium constant, $K_d$, and rate constant, $k_{off}$, of protein-DNA complex dissociation. We found that nonionic surfactants worked best: they lyse the plasma membrane without significantly influencing $K_d$, $k_{off}$, or the EOF. This work creates the foundation for studies of protein-DNA interactions in single cells by chemical cytometry.

**Keywords:** Capillary electrophoresis / Cell lysis / Chemical cytometry / Protein-ligand interaction / Surfactant DOI 10.1002/elps.200500732

## 1 Introduction

Noncovalent protein-DNA interactions play a crucial role in DNA replication, DNA integrity control, DNA damage repair, and many other cellular processes [1–3]. Studying such interaction in single cells is important for understanding regulatory processes, which lead to the formation of heterogeneous cell populations in embryogenesis, carcinogenesis, stem cell proliferation, neurodegeneration, *etc.* Fluorescence resonance energy transfer (FRET) in combination with advanced microscopy has been successfully used for studying protein-protein interactions for a long time [4–6]; however, to the best of our knowledge, it has never been applied to protein-DNA interactions. The most likely reason of this is that DNA is a very poor fluorophore and it is difficult to place a fluorophore on native DNA. This work was inspired by the insight that chemical cytometry may be a suitable technique for investigating protein-DNA interactions in single cells, as it can facilitate the use of exogenous fluorescently labeled DNA.

Cytometry is a collective name for techniques used in studies of single cells. The three major cytometry techniques are image cytometry, flow cytometry, and chemical cytometry. Chemical cytometry utilizes highly efficient capillary separation tools of instrumental analytical chemistry to analyze chemical contents of individual cells.

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**Abbreviations:** **CYMAL-2**, 2-cyclohexyl-1-ethyl-β-D-maltoside; **DDM**, *n*-decyl-β-D-maltopyranoside; **D-DDM**, *n*-dodecyl-β-D-maltopyranoside; **FBS**, fetal bovine serum; **fDNA**, fluorescein-labeled ssDNA; **FS-12**, *n*-dodecylphosphocholine; **GFP**, green fluorescent protein; **HEGA-10**, decanoyl-*N*-hydroxyethyl glucamide; **LDAO**, lauryldimethylamine-*N*-oxide; **NECEEM**, nonequilibrium CE of equilibrium mixtures; **OCTG**, *n*-octyl-β-D-glucopyranoside; **SSB**, ssDNA binding protein

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Conceptually, in chemical cytometry (i) a single cell is injected into a capillary, (ii) lyzed inside the capillary to solubilize the cellular components, and (iii) the cellular contents are separated by CE or chromatography and detected by fluorescence or electrochemistry. Dovichi and coworkers [7, 8] introduced the term of chemical cytometry in 2000, but the approach of capillary separation of single-cell contents has been known for as long as five decades [9–13]. Dovichi and Hu [14] have recently reviewed chemical cytometry; a few important works however appeared after that review. Ramsey and coworkers [15, 16] developed microfluidic chips for chemical cytometry. Chemical cytometry on chips can facilitate higher throughput of analyses. Our group introduced two-channel chemical cytometry that analyzes daughter cells simultaneously [17]. Two-channel chemical cytometry revealed asymmetry between sister cells in a cancer cell line. The latter approach makes it possible to study in detail molecular mechanisms of asymmetric cell division by chemical cytometry.

There are two major ways of lysing cells in chemical cytometry: outside the capillary [18] and inside the capillary [8, 19]. Lysing outside the capillary is performed by a laser-induced shock wave. The laser pulse is synchronized with a pulse of suction so that cell lysate is injected into the capillary within ~30 ms after the laser pulse. Out-of-capillary cell lysis has the advantage of allowing one to work with adherent cells. The disadvantages of this approach are the requirement for sophisticated and expensive laser equipment and the danger of incomplete injection of the cell lysate. Lysis inside the capillary is performed after the intact cell is injected. It is simpler, less expensive in instrumentation, and guarantees no losses of cellular components. At the down side, the injection of the intact cell requires that the cell should not be attached to the surface. This can be achieved by using surfaces, to which cells do not adhere, or by using suspended cells such as hemopoietic stem cells. Four techniques of cell lysis inside the capillary have been described: (i) ultrasonic treatment [20], (ii) high-electric field [16, 19], (iii) low-ionic-strength solution [21], and (iv) a surfactant [8, 17, 22]. Using a surfactant is a simple and efficient way. Cell lysis was demonstrated using a surfactant in the run buffer [8, 17] and by a plug of a surfactant injected after the cell [22]. If a surfactant is used to lyse cells in chemical cytometry of protein-DNA interactions, it must not affect the stability of protein-DNA complexes.

This work aimed at finding surfactants and conditions, which could serve the application of chemical cytometry in studying protein-DNA interactions. We studied a panel of ten surfactants of three types: ionic, zwitterionic, and nonionic. All of them can lyse cells. Initially, concentrations of surfactants were selected at which cell lysis on a microscope slide takes approximately 0.5 and 1 min. These concentrations of surfactants were then examined for cell lysis in the capillary. Finally, the effect of the lysing concentrations of surfactants on complexes between ssDNA binding protein (SSB) and ssDNA was tested using nonequilibrium CE of equilibrium mixtures (NECEEM) [23–30]. NECEEM uniquely allows measurements of the equilibrium constant, $K_d$, and rate constant, $k_{off}$, of complex dissociation from a single experiment. This investigation revealed that nonionic surfactants satisfy the condition of efficiently lysing cell membrane while only slightly affecting protein-DNA complex stability. Zwitterionic surfactants do not appear to destroy protein-DNA complex but they can coat the inner surface of the capillary. The latter can change the EOF and result in either significant variations in migration times of peaks or the need to use the reverse polarity (negative electrode at the injection end). Because of this, zwitterionic surfactants are less practical in chemical cytometry studies of protein-DNA interactions. Ionic surfactants are widely known to denature proteins. Due to this property, they destroy the protein-DNA complex. Ionic surfactants are thus not suitable for studies of protein-DNA interactions by chemical cytometry. This work makes the first step toward studies of protein-DNA complexes in single cells by chemical cytometry. It will also facilitate chemical cytometry-based quantitative affinity analyses of proteins with DNA aptamers as affinity probes.

## 2 Materials and methods

### 2.1 Reagents and materials

PBS, DMEM, fetal bovine serum (FBS), antibiotic mixture, and trypsin were purchased from Invitrogen Life Technologies (Invitrogen, Burlington, ON). Fluorescein-labeled ssDNA (fDNA, 5'-fluorescein-GCGGAGCGTGGCAGG) was kindly donated by Dr. Yingfu Li (McMaster University, Hamilton, ON). Seven surfactants: decanoyl-*N*-hydroxyethylglucamide (HEGA-10), *n*-octyl-β-D-glucopyranoside (OCTG), *n*-dodecylphosphocholine (FS-12), 2-cyclohexyl-1-ethyl-β-D-maltoside (CYMAL-2), lauryldimethylamine-*N*-oxide (LDAO), *n*-dodecyl-β-D-maltopyranoside (D-DDM), and *n*-decyl-β-D-maltopyranoside (DDM) were from a surfactant kit produced by Anatrace Inc. (Maumee, OH). All other reagents, including surfactants $t$-Oct-$C_6H_4$-$(OCH_2CH_2)_{9-10}OH$ (Triton X-100), CHAPS, and SDS were purchased from Sigma-Aldrich (Oakville, ON). Bare fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ) and used unmodified.

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### 2.2 Cell culture

The Madin Darby canine kidney (MDCK) cell line with green fluorescent protein (GFP) stably expressed was used in this work. Cells were grown to 80–90% confluence in DMEM, supplemented with 10% heat-inactivated FBS, 50 U/mL penicillin, and 50 U/mL streptomycin at 37°C in 5% $CO_2$ atmosphere. Single-cell suspension ($10^6$/mL) was prepared in PBS.

### 2.3 Cell lysis conditions

Ten microliters of cell suspension was mixed with 10 μL of varying concentrations of different surfactants (in 50 mM Tris-HCl buffer at pH 8.2) on a microscopic slide. The cells were then viewed under an Olympus IX-71 inverted microscope (Carson Group, Markham, ON). Cell lysing was assessed by visually following the lost of the integrity of the cell membrane for over 90% of cells under continuous observation. The concentrations of surfactants that lysed cells within approximately 0.5 and 1 min were selected for further analysis.

### 2.4 Cell lysis inside the capillary

Cell lysis experiments within a capillary were conducted with a CE-based chemical cytometer described in detail elsewhere [8]. In order to examine the efficiency of cell lysis inside the capillary, surfactants were added to the run buffer (50 mM Tris-HCl buffer at pH 8.2) at concentrations selected on a microscope slide (see Section 2.3). In this part of the work, we used a capillary of the following dimensions: length of 60 cm, id of 20 μm, and od of 150 μm. The capillary was prefilled with the run buffer. At the beginning of each run, the capillary was rinsed by 0.1 M HCl, 0.1 M NaOH, deionized water, and the run buffer for 2 min each. A single GFP-expressing MDCK cell was injected into the capillary from PBS by a 2 s pulse of suction. The cell was incubated in the capillary for 1 min to allow the surfactant in the run buffer to diffuse toward the cell and lyse it. Finally, the cell lysate was moved to the detection end by a pressure of 60 psi. The peak of GFP was detected and its shape was a determining parameter of whether or not cell lysis was efficient: a nonlysed cell generates a spike, while a lysed cell results in a smooth peak.

### 2.5 Effect of surfactants on protein-DNA complex

We used NECEEM to study the effect of surfactants on protein-DNA complex stability. Experiments were conducted with a P/ACE MDQ apparatus with LIF detection (Beckman-Coulter, Fullerton, CA). Fluorescence of fDNA was excited by the 488 nm line of an argon-ion laser (5 mW). In this part of the work, we used a capillary of the following dimensions: length of 40 cm, id of 75 μm, and od of 365 μm. Electrophoresis was run with a positive electrode at the injection end and an electric field of 600 V/cm. The equilibrium mixture was prepared by incubating for 15 min a solution of 250 nM SSB/200 nM fDNA in 50 mM Tris-HCl buffer at pH 8.2 with each surfactant at the concentration selected above. Prior to each run, the capillary was rinsed consequently with 0.1 M HCl, 0.1 M NaOH, deionized water, and the run buffer for 2 min each. The samples were injected into the capillary by a pressure pulse of 0.5 psi × 5 s. The solution of each surfactant in 50 mM Tris-HCl at pH 8.2 with the concentration selected above was used as a run buffer. The areas and the migration times of the peaks of the SSB-fDNA complex and free fDNA and the area of fDNA dissociated from the SSB-fDNA complex were found from electropherograms and used to determine the equilibrium constant, $K_d$, and rate constant, $k_{off}$, of complex dissociation. Data collection, processing, and analysis were performed using the Beckman 32 Karat Software Version 5.0. $K_d$ and $k_{off}$ of each surfactant were calculated using the following equations [28–30]:

$$K_d = \frac{[SSB](1 + A_1/(A_2 + A_3)) - [fDNA]}{1 + (A_2 + A_3)/A_1} \quad (1)$$

and

$$k_{off} = \ln\left(\frac{A_2 + A_3}{A_2}\right)/t_{SSB \cdot fDNA} \quad (2)$$

where $A_1$, $A_2$, and $A_3$ are the areas of peaks of fDNA, SSB-fDNA complex, and fDNA dissociated from the complex, respectively, and $t_{SSB \cdot fDNA}$ is the migration time of the SSB-fDNA complex.

## 3 Results and discussion

### 3.1 Cell lysis

First, we determined minimum concentrations of surfactants, which lyse cells in cell suspension on a microscope slide. All surfactants used in this work could lyse cells gently by incorporating themselves into the cellular membrane. The loss of the intact shape of the plasma membrane was our criterion of cell lysis. Two different concentrations for each surfactant were selected for further study: one that lyses the cells in approximately 1 min and another one that is sufficient to lyse cells in approximately 0.5 min (Table 1).

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**Table 1.** Effect of ten surfactants on the stability of the protein-DNA complex

| Surfactant and its nature | Concentration of the surfactant (% v/v) | Time required for cell lysis (min) | Effect on protein-DNA interaction studied with NECEEM | |
|---|---|---|---|---|
| | | | $K_d$ (nM) | $k_{off}$ (/s) |
| No surfactant | 0 | Cells are not lysed | 41 ± 21 | 2.1 ± 0.6 |
| SDS anionic | 0.1 | ~0.2 | Not applicable: destroys the protein-DNA complex | |
| FS-12 | 0.1 | ~0.5 | Not applicable: poorly interpretable NECEEM electropherogram | |
| Zwitterionic | 0.05 | ~1.0 | | |
| LDAO | 0.5 | ~0.5 | Not applicable: poorly interpretable NECEEM electropherogram | |
| Zwitterionic | 0.25 | ~1.0 | | |
| CHAPS | 1 | ~0.5 | 64 ± 25 | 0.22 ± 0.15 |
| Zwitterionic | 0.5 | ~1.0 | 22 ± 5 | 0.24 ± 0.05 |
| HEGA-10 | 1 | ~0.5 | 21 ± 6 | 2.7 ± 0.7 |
| Nonionic | 0.5 | ~1.0 | 21 ± 7 | 2.4 ± 0.6 |
| OCTG | 1 | ~0.5 | 29 ± 8 | 1.2 ± 0.2 |
| Nonionic | 0.5 | ~1.0 | 27 ± 2 | 1.2 ± 0.2 |
| Triton X-100 | 0.1 | ~0.5 | 30 ± 9 | 1.5 ± 0.4 |
| Nonionic | 0.05 | ~1.0 | 57 ± 38 | 1.9 ± 0.9 |
| CYMAL-2 | 1 | ~0.5 | 14 ± 2 | 1.6 ± 0.8 |
| Nonionic | 0.5 | ~1.0 | 27 ± 2 | 1.0 ± 0.2 |
| D-DDM | 1 | ~0.5 | 37 ± 19 | 1.0 ± 0.5 |
| Nonionic | 0.5 | ~1.0 | 47 ± 15 | 1.6 ± 0.7 |
| DDM | 0.5 | ~0.5 | 69 ± 3 | 1.6 ± 0.4 |
| Nonionic | 0.25 | ~1.0 | 75 ± 5 | 1.8 ± 0.7 |

Second, we examined cell lysis inside the capillary. In the absence of a surfactant in the run buffer the cell was not lysed: an intact cell passed through the detector. The time of cell's passing the laser beam was less than 10 ms; as a result, intact cells generated very short spikes (Fig. 1A). When a surfactant was present in the run buffer at a lysing concentration, the cell was lysed and the cellular lysate passed the detector as a well-dispersed homogeneous solution. A typical wide peak of solubilized GFP was detected (Fig. 1B). Using this criterion we could monitor if a cell was lysed within a capillary or not. Ten surfactants that we selected were examined for cell lysis inside a capillary in a chemical cytometer. This part of our experiment proved that all ten surfactants at selected concentrations could lyse cells effectively within the capillary. They all were further examined for their influence on the stability of the protein-DNA complex.

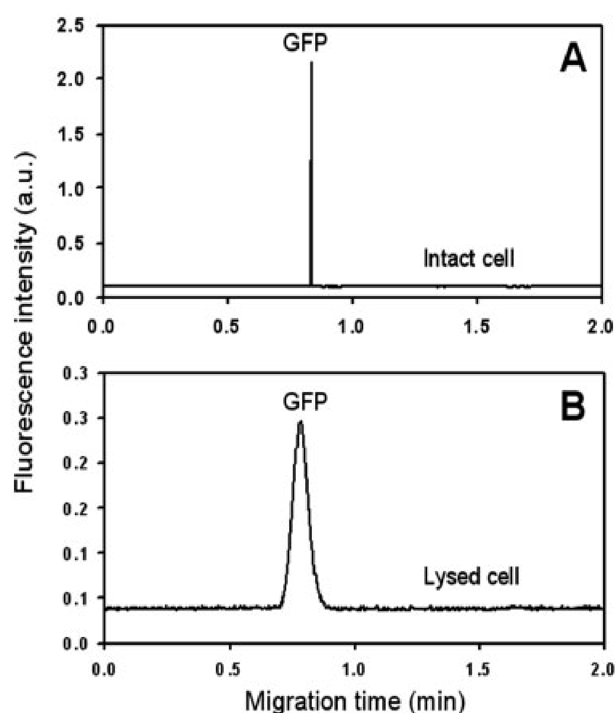


**Figure 1.** Fluorescent signals of GFP from an intact cell (A) and a lysed cell (B).

## 3.2 Effects of surfactants on the protein-DNA complex stability

In order to examine the effects of surfactants on protein-DNA interactions, we used the interaction between SSB from *Escherichia coli* and fDNA as a model. The effect of

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surfactants on SSB-fDNA interaction was examined using the NECEEM method. The equilibrium mixture of SSB with fDNA was prepared in the run buffer and sampled for the NECEEM analysis. The run buffer could contain one of the ten surfactants; in control experiments, we used the run buffer without a surfactant in it. The NECEEM experiments were carried out with a P/ACE MDQ apparatus with LIF detection. Concentrations of SSB and fDNA, 250 and 200 nM, respectively, were chosen to obtain approximately equal peak areas for free fDNA and SSB-fDNA complex. To minimize the dissociation of the complex during the analysis we shortened the migration time by selecting a relatively high-electric field of 600 V/cm and a relatively short capillary of 40 cm. Under these conditions electrophoresis generally took less than 15 min. The optimized conditions were used to study the influence of surfactants on the stability of the SSB-fDNA complex.

In the absence of a surfactant in the run buffer we observed a typical NECEEM electropherogram (Fig. 2C). The interpretation of the NECEEM electropherogram was explained in detail elsewhere [28–30]. It consisted of a peak of free fDNA, a peak of the intact SSB-fDNA complex, and an exponential smear of fDNA, which dissociated from the complex during the separation. The areas of these three features ($A_1$, $A_2$, and $A_3$, respectively) and the migration time of the SSB-fDNA complex ($t_{SSB \cdot fDNA}$) were used to calculate $K_d$ and $k_{off}$ (using equations 1 and 2) for the "no-surfactant" control.

In the presence of the ionic surfactant SDS, no peak of the SSB-fDNA complex or smear of fDNA dissociated from the complex was observed (Fig. 2A). This indicates that no detectable amount of SSB-fDNA complex was present in the equilibrium mixture. SDS and other ionic surfactants are known to denature proteins [31, 32]. Denaturing the protein destroys the protein-DNA complex. We, then, concluded that ionic surfactants are, in general, not suitable cell-lysing agents for studies of protein-DNA interactions at the single-cell level by chemical cytometry.

Zwitterionic surfactants resulted in either the elongation of the migration times of the peaks as was observed for CHAPS or the inability to detect the peaks as was observed for FS-12 and LDAO. Our observations are explained by the ability of zwitterionic surfactants to coat the inner surface of the capillary, and as a result, they suppress the EOF or even reverse it [33–35]. CHAPS suppresses EOF while both FS-12 and LDAO apparently reverse it. To examine the latter suggestion we used FS-12 and LDAO in NECEEM experiments with the reversed polarity (Fig. 2B). We have observed the peak of the free fDNA for both surfactants. FS-12 and LDAO are thus not practical as cell-lysing agents. CHAPS can potentially be

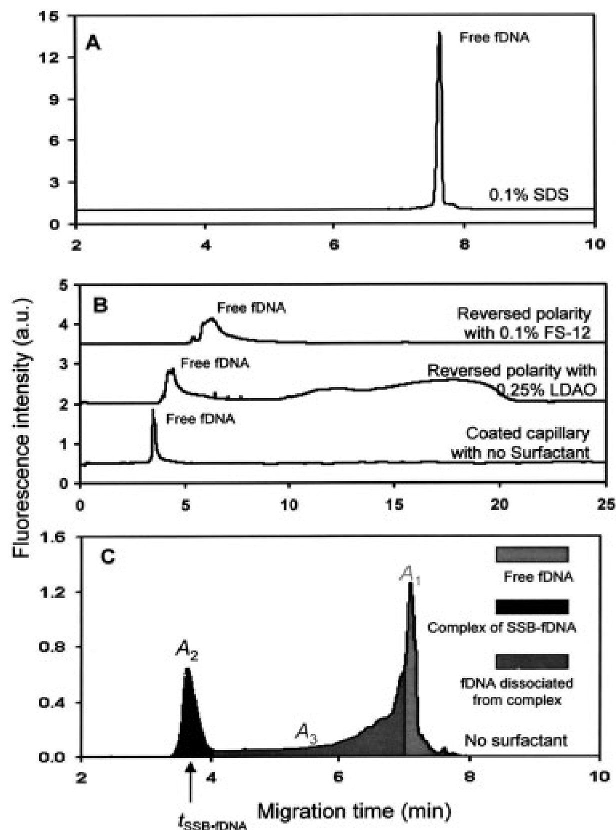


**Figure 2.** Effect of (A) ionic and (B) zwitterionic surfactants on SSB-fDNA complex stability. (C) Control experiment: NECEEM electropherogram of SSB-fDNA system in the absence of surfactants. Colored areas, $A_1$, $A_2$, and $A_3$, and the migration time of the protein-DNA complex, $t_{SSB \cdot fDNA}$, were used in Eqs. (1) and (2) for the determination of $K_d$ and $k_{off}$.

used to lyse cells in chemical cytometry of protein-DNA interactions. However, its ability to influence EOF encourages the search for alternatives.

Finally we studied the influence on SSB-fDNA complex stability of six nonionic surfactants: HEGA10, OCTG, Triton X-100, CYMAL-2, D-DDM, and DDM. Nonionic surfactants are used in order to solubilize membrane proteins for their further structural studies by NMR or MS [36–38]. We did not observe significant changes in NECEEM electropherograms upon adding nonionic surfactants to the system; Fig. 3 shows NECEEM electropherograms for six nonionic surfactants. Nonionic surfactants did not change significantly $K_d$ and $k_{off}$ values of protein-DNA interaction (see Table 1). They slightly suppressed EOF but the standard capillary reconditioning procedure was sufficient for obtaining reproducible NECEEM electropherograms. Nonionic surfactants are thus suitable and preferable cell-lysing agents in studies of protein-DNA interaction by chemical cytometry.

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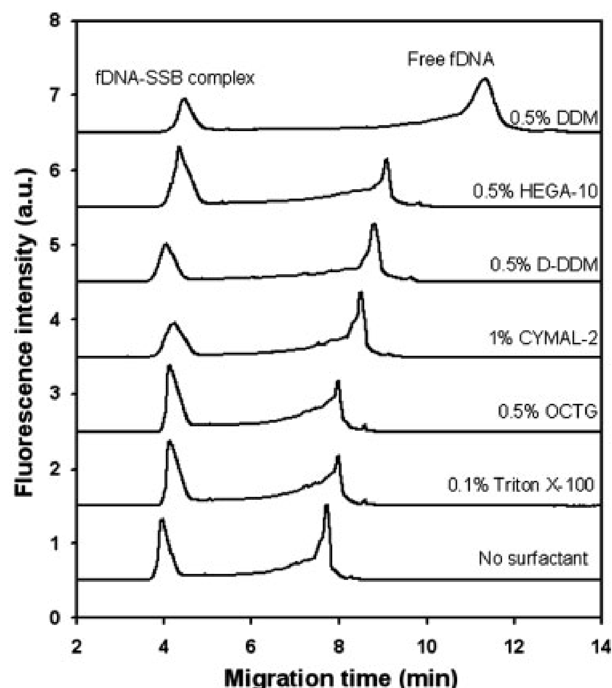


**Figure 3.** Effect of nonionic surfactant, HEGA10, OCTG, Triton X-100, CYMAL-2, D-DDM, and DDM, on the stability of the SSB-fDNA complex.

## 4 Concluding remarks

Chemical cytometry is an attractive tool for studies of protein-ligand interactions (*e.g.*, protein-DNA, protein-protein, protein-small molecule) at the single-cell level. Nondenaturing cell lysis is a necessary step in such studies. In this work, we screened surfactants of three classes: ionic, zwitterioninc, and nonionic for their ability to lyse cells while not destroying protein-DNA complexes. We found that nonionic surfactants worked best as nondenaturing cell-lysing agents, which do not deteriorate the quality of CE separation. In addition to the listed applications, these lysing agents can be also used in aptamer-based quantitative affinity analyses of proteins at the single-cell level by chemical cytometry.

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