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Minimizing adsorption of histidine-tagged proteins for the study of protein–deoxyribonucleic acid interactions by kinetic capillary electrophoresis

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ABSTRACT

Affinity interactions between DNA and proteins play a crucial role in many cellular processes. Kinetic Capillary Electrophoresis is a highly efficient tool for kinetic and equilibrium studies of protein–DNA interactions. Recombinant proteins, which are typically used for in vitro studies of protein–DNA interactions, are often expressed with a His tag to aid in their purification. In this work, we study how His tags affect Kinetic Capillary Electrophoresis analysis of protein–DNA interactions. We found that the addition of a His tag can increase or decrease protein adsorption to a bare-silica capillary wall, dependent on the protein. For Kinetic Capillary Electrophoresis measurements, it is essential to have as little protein adsorption as possible. We screened a number of capillary coatings to reduce adsorption of the His-tagged DNA mismatch repair protein MutS to the capillary wall and found that UltraTrol LN was the most effective coating. The effectiveness of the coating was confirmed with the prevention of adsorption of His-tagged fat mass and obesity-associated protein. Under typical conditions, the coating reduced protein adsorption to a level at which accurate Kinetic Capillary Electrophoresis analysis of protein–DNA interactions was possible. We further used Kinetic Capillary Electrophoresis to study how the His tag affected K_d of protein–DNA interactions for the MutS protein. Using UltraTrol LN, we found that the effect of the His tag was insignificant.

1. Introduction

Proteins can selectively bind DNA forming stable non-covalent complexes [1]. These interactions are involved in many important cellular processes such as DNA replication, repair, modification, communication, regulation, and genome arrangement [2,3]. The simplest mechanism for a protein–DNA interaction is the following:

$$P + DNA \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} P \bullet DNA \quad K_d = \frac{k_{off}}{k_{on}} \tag{1}$$

This mechanism describes binding of one protein molecule (P) with one DNA molecule. The rate constant of complex (P·DNA) formation is denoted by k_{on} and the rate constant of complex dissociation is denoted by k_{off}. The equilibrium dissociation constant, K_d, can be presented as a ratio between k_{off} and k_{on}. Kinetic and equilibrium studies which determine these parameters are important as they allow us to understand the dynamics of protein–DNA interactions. Once such information is obtained, it can be applied towards understanding cellular processes, incorporated into drug screenings and utilized for biotechnological applications [4].

Kinetic studies allow for the determination of k_{on} and k_{off}, which, in turn, define K_d. Most kinetic methods require the use of a solid surface for the separation of protein or DNA components. Examples of such methods include electrophoretic mobility shift assays, filter binding assays, Differential Radial Capillary Action of Ligand Assay, and Surface Plasmon Resonance [5–8]. Since these techniques require a solid surface during separation, they have several common drawbacks. The performance of these techniques can be affected by non-specific interaction of reaction components with the solid surface [9]. If reaction components need to be immobilized to the solid surface, such as with SPR, their native conformation and properties can be altered [10]. Immobilization of the protein or DNA takes additional time and incurs additional costs. Finally, all prior techniques are difficult to model mathematically due to the complexity of surface–solution interactions [9]. Due to these reasons, the kinetic and equilibrium parameters obtained from the above techniques can be prone to errors.

Kinetic capillary electrophoresis (KCE) refers to a toolbox of kinetic, solution-based affinity methods used to study biomolecular interactions. To date, KCE has been applied towards studying protein–oligonucleotide, protein–peptide, protein–small

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molecule, and oligonucleotide–small molecule interactions [11–14]. All KCE techniques involve the separation of interacting species using capillary electrophoresis (CE) at near-physiological conditions. Separation of various biomolecular species in an electric field occurs due to their unique size-to-charge ratios. KCE techniques are applied to studying protein–DNA interactions because the unbound DNA, unbound protein, and protein–DNA complex are easily separated without a gel. When KCE analysis is performed, either the DNA or the protein species is labelled with a fluorophore and detected using laser induced fluorescence (LIF) [15]. The resulting electropherograms reveal the fractions of unbound DNA (or unbound protein) and the protein–DNA complex. By determining peak areas and migration times in a KCE electropherogram, it is possible to determine k_{on}, k_{off}, and K_d. Unlike the surface-based techniques, KCE facilitates studies of biomolecular interactions without the need to immobilize the protein or DNA. Therefore, protein–DNA interaction studies using KCE do not face the drawbacks of the surface-based methods discussed above [9].

A key requirement for KCE is that neither the protein nor the DNA interacts with the capillary surface. This requirement is often violated by proteins [16]. During KCE analysis, separations are typically performed in run buffers with low salt concentration and pH of 7–9 [17]. Under these conditions, the capillary surface is negatively charged as the silanol groups of the glass capillary have pK_a values of 3.5–4 [18]. Positively charged proteins or electropositive regions of proteins in general can readily interact with the negatively charged capillary surface [19]. Adsorption is detrimental for KCE due to several reasons. Firstly, adsorption can prevent protein and the protein–DNA complex from reaching the detector [20]. Since the loss of analytes is difficult to predict, the kinetic and equilibrium parameters derived from KCE will be inaccurate. Secondly, adsorbed proteins can lead to changes in the electroosmotic flow (EOF) and can cause peak asymmetry due to tailing [21]. Both of these effects reduce the separation efficiency and the resolution between unbound DNA, unbound protein, and the protein–DNA complex [22]. Due to the reasons above, protein adsorption makes it difficult to apply simplified mathematical techniques typically used in KCE analysis. This reduces the applicability of KCE towards studying protein–DNA interactions.

Due to the frequent occurrence of protein adsorption during KCE analysis, it is necessary to have a technique to measure protein adsorption. There are methods that quantify protein adsorption during CE, however, they are time intensive and require specialized equipment. For example, the method established by Towns and Regnier requires a specialized instrument with dual detectors on the capillary to determine the degree of protein adsorption [23]. The method established by Verzola et al. requires an overnight incubation and gradual desorption of the protein adsorbed to the capillary surface. Both the incubation and desorption take a long period of time, and therefore, the technique cannot be easily applied towards screening multiple proteins [24].

We recently introduced an easier alternative for measuring protein adsorption, which utilizes pressure propagation of the protein through the capillary [17]. In this method, a plug of fluorescently labelled protein is injected into the capillary and propagated at a low pressure towards the detection window. Analytes that do not adsorb onto the capillary surface show symmetrical peaks, while analytes that do adsorb exhibit peak asymmetry. Using this technique, only the fraction of proteins which elute without adsorbing onto the capillary surface can be detected [17].

Herein, the pressure propagation technique was modified to visualize both the protein which elutes under the propagation conditions and the protein which adsorbs onto the capillary surface. The adsorbed protein was visualized by flushing the capillary with strong base, causing protein desorption. This technique allowed us to measure the adsorption of recombinant proteins.

Affinity-purified recombinant proteins are typically used in in vitro studies. To assist purification, affinity tags such as GST, MBP, and FLAG-tags are incorporated in protein sequences [25]. Affinity tags are used under the assumption that they do not significantly alter the native properties of proteins [26]. There is, however, increasing evidence that they can change native protein properties such as protein structure, enzymatic activity, substrate specificity, and binding affinities [27,28]. Of all the tags currently available, His tags are the most widely used due to their small size and the simplicity of purification with immobilized metal affinity columns [25]. They are typically composed of six repetitive histidine residues which are inserted into the C or N terminus of a protein [27,29] His tags can be removed using targeted proteolysis with peptidases such as aminopeptidase dipeptide I, thrombin, and enterokinase. However, enzymatic cleavage can have adverse effects as it can also hydrolyze sites on the primary amino acid sequence of the protein and render it inactive [30–32]. Due to this reason His-tagged proteins are most often studied without removing the tag.

To date, KCE has not been applied towards the study of His-tagged proteins. Since affinity tags have been shown to alter the native properties of proteins, the goal of this study was to understand if His-tagged proteins require a special consideration in KCE. We studied the influence of His tags on protein adsorption with bare-silica capillary walls for three DNA binding proteins: DNA mismatch repair protein MutS (MutS), fat mass and obesity associated protein (FTO) and alpha-ketoglutarate-dependent dioxygenase alkB homologue 3 (ABH3). The His tag was found to alter protein's native adsorption properties. We have shown that His tags can cause a non-adsorbing protein (MutS) to adsorb and an adsorbing protein (ABH3) to not adsorb onto the bare-silica capillary wall. The first case is detrimental for KCE measurements while the second one is beneficial. We focused on finding a coating that could reduce the level of adsorption of His-tagged proteins and could facilitate kinetic studies of protein–DNA interactions by KCE. We tested a number of capillary coatings and found that UltraTrol LN was the most effective coating for preventing adsorption for His-tagged MutS protein. This coating allowed KCE-based kinetic measurements for the interaction between DNA and His-tagged MutS (His-MutS) protein. The effectiveness of UltraTrol LN coating was further confirmed by studying His-tagged FTO (His-FTO) protein. This study was the first to measure the equilibrium kinetics of His-tagged proteins with KCE.

2. Materials and methods

2.1. Materials

Untreated fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ). Ultratrol LN, a polyacrylamide-based capillary coating was purchased from Target Discovery (Palo Alto, CA). CElixer, a dynamic coating, was purchased from Micro-Solv Technology Corporation (Eatontown, NJ). Non-tagged MutS was expressed in our laboratory using *Escherichia coli* expression systems. His-FTO, ABH3, and His-tagged ABH3 (His-ABH3) were kindly provided by Christopher Schofield (University of Oxford, UK). His-MutS was purchased from GenScript (Piscataway, NJ). A synthetic DNA library with a 40-nt random region and 20-nt primer regions was purchased from Integrated DNA Technologies (Toronto, ON, Canada). The library contained a 5′ FAM label to enable LIF detection. A MutS aptamer (5′ – FAM - CTT CTG CCC GCC TCC TTC CTG GTA AAG TCA TTA ATA GGT GTG GGG TGC CGG GCA TTT CGG AGA CGA GAT AGG CGG ACA CT - 3′) was also ordered from

Integrated DNA Technologies and used in Nonequilibrium capillary electrophoresis of equilibrium mixtures (NECEEM).

All solutions used were made using double distilled water deionized with Milli-Q and filtered through a 0.22-μm filter (Millipore, Nepean, ON, Canada). All reagents were purchased from Sigma–Aldrich, unless specified. Trizma base, sodium tetraborate, sodium dihydrogen phosphate and sodium bicarbonate were used to prepare buffers at various pH levels. Buffer pH was adjusted with glacial acetic acid, phosphoric acid, or sodium hydroxide (Caledon Labs, Georgetown, ON, Canada). Magnesium chloride for the incubation buffer was also purchased from Caledon Labs. Chromeo P503 pyrylium dye was purchased from Active Motif (Burlington, ON, Canada) for protein labelling.

2.2. Instrumentation

All experiments were performed using a PACE/MDQ CE-Instrument (Beckman-Coulter, Fullerton, CA). A 488-nm solid-state laser (JDSU, Santa Rosa, CA) was used to excite fluorescence of the Chromeo label on the protein and FAM label on the DNA. Two band-pass filters, centred at 520 and 610 nm, were used to detect FAM and Chromeo labels, respectively.

2.3. PVA coated capillary preparation

A 130-cm long uncoated, fused silica capillary was preconditioned by flushing for 1 h at 12 psi (83 kPa) with 1 M NaOH and then with H_2O. PVA (5% w/v) solution was prepared in boiling H_2O until a clear solution was obtained. The capillary was flushed with the PVA solution at 15 psi (103 kPa) for 10 min and then purged with nitrogen gas at 10 psi (69 kPa) until all liquid was removed from the capillary. The capillary was then purged with nitrogen gas at 5 psi (35 kPa) while being dried in a 140 °C oven overnight. The capillary was cut into 50-cm long segments and a window was burned 10 cm from capillary end using boiling sulfuric acid.

2.4. Protein labelling

A Chromeo working solution was made by a 1:100 dilution of the 25 mM Chromeo P503 dye in 0.1 M sodium bicarbonate buffer. All proteins were subsequently diluted 1:2 in the Chromeo working solution. The protein was incubated overnight in a 4 °C, dark environment for the labelling reaction to be completed. All samples were further diluted in 0.1 M sodium bicarbonate buffer.

2.5. Calculation of pI

The FASTA sequence of MutS from *Thermus aquaticus* (Q56215), ABH3 (Q96Q83) and human FTO (Q9C0B1) were obtained from UniProtKB database. The theoretical p*I* values of the proteins were calculated by entering the FASTA sequences into the p*I* computing tool provided by ExPASY.

2.6. Experimental conditions

All capillaries used for experimentation were 50 cm in length and had an inner diameter of 75 μm. Bare capillaries were preconditioned with methanol, 0.1 M HCl, 0.1 M NaOH, H_2O and 50 mM tris–acetate (pH 8.3) at 40 psi (276 kPa) for 5 min each. To apply the CElixer coating, the capillary was rinsed with 0.1 M NaOH for 5 min at 30 psi (207 kPa) followed by H_2O at 2 min for 30 psi (276 kPa). The initiator and accelerator solutions supplied with the CElixer kit were applied at 20 psi (138 kPa) for 1 and 2 min, respectively. UltraTrol LN capillary was prepared by applying 1 M NaOH, H_2O, UltraTrol LN solution and 50 mM tris–acetate buffer, each at 20 psi (138 kPa) for 2.5 min. All PVA-coated and LPA-coated capillaries were preconditioned with H_2O and 50 mM tris–acetate for 8 min prior to use.

A sample plug was injected at 0.5 psi (4 kPa) for 5 s in all experimental conditions. When using PVA- and LPA-coated capillaries, the sample was propagated 10 cm from the capillary outlet to the detector at 0.5 psi in 50 mM tris–acetate. Strong base was not used with these capillaries because the coatings become unstable for further use. In all other experimental conditions, the sample was first propagated to the detector at 0.5 psi in 50 mM sodium phosphate (pH 6.0), 50 mM tris–acetate (pH 8.3) or 25 mM borax (pH 9.3). All adsorbed protein was then removed from the capillary wall by 1 M NaOH at 0.5 psi (4 kPa) until the sample plug reached the detector. Both CElixer and UltraTrolLN coatings were reapplied after the use of 1 M NaOH. When using the PVA- and LPA-coated capillaries, adsorption was assessed by the peak symmetry. In all other propagation conditions, the ratio of eluted protein versus adsorbed protein was calculated by integrating the peak areas of the analyte under each propagation condition.

2.7. Determining K_d

K_d values for MutS and His-MutS interaction with MutS aptamer were determined in a bare capillary and in an UltraTrol LN-coated capillary. The MutS aptamer was folded into the correct conformation by heating the sample to 95 °C for 1 min and then cooling it to 25 °C at a rate of 0.5 °C/s. In order to calculate K_d through NECEEM, 10 nM of internal standard (fluorescein), 100 nM of protein and 50 nM of aptamer were incubated and propagated using the conditions mentioned above. Experiments for each unique condition were repeated three times. The equilibrium dissociation constants were determined by using the following equation:

$$K_d = \frac{[T]_0 - [L]_0\left(1 - \frac{A_L}{A_C+A_D+A_L}\right)}{\left(\frac{A_C+A_D+A_L}{A_L} - 1\right)} \quad (2)$$

where, $[T]_0$ and $[L]_0$ refer to initial protein and DNA concentrations; A_L, A_C, A_D refer to the integrated areas corresponding to the unbound ligand, intact complex, and dissociated complex, respectively. A two-tail *t*-test was performed to determine statistical difference between K_d values ($p > 0.05$).

3. Results and discussion

3.1. Criteria for assessing analyte adsorption

The effectiveness of each technique in preventing protein and DNA adsorption was assessed by pressure propagating each analyte through the capillary first in a run buffer and then desorbing any adsorbed components with a strong base. The peak shape of a sample which has been injected and propagated by pressure is dependent upon the ability of the analytes to remain in solution and migrate with the pressure-driven flow. When a sample is both injected and propagated by pressure, the anterior and the posterior of the plug will display parabolicity [33]. In the absence of protein adsorption, the parabolic sample plug should be detected as a symmetrical peak.

If analytes interact with the capillary surface, the peak shape can show broadening, tailing, or baseline elevation. Peak broadening occurs because of fast adsorption and desorption processes on the capillary surface [34]. Peak tailing occurs due to fast adsorption and either slow or negligible desorption processes on the capillary surface [33]. Baseline elevation can occur due to adsorption of the analyte throughout the capillary, followed by a gradual desorption. The amount of adsorbed analytes can be estimated by pressure propagating a strong base through the capillary. Since the pH of

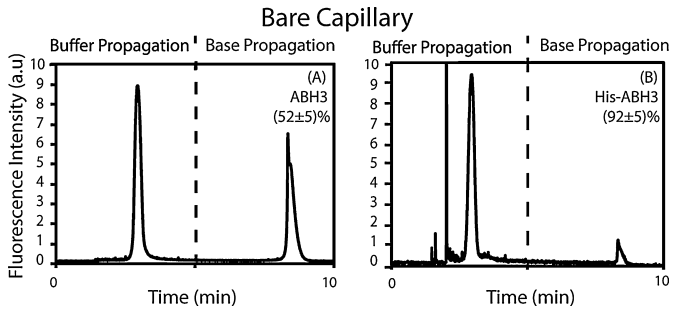

Fig. 1. Assessment of adsorption of (A) 2 μM ABH3 and (B) 2 μM His-ABH3 in a bare capillary. Analyte is first propagated in 50 mM tris–acetate buffer and then propagated in 1 M NaOH. The dotted line divides the two different propagation conditions. The percentage of analyte eluted with the buffer is indicated on the top-right corner of each electropherogram. Each experiment was performed in triplicate to calculate the average fraction of protein which elutes with buffer. Standard error of mean was used to determine statistical difference between each propagation condition.

1 M NaOH is much higher than the p*I* of proteins, the protein will become deprotonated and lose any positive charge. This prevents electrostatic interactions with the wall, allowing the adsorbed protein to dissociate and reach the detector with the pressure-driven flow.

3.2. Adsorption in a bare capillary

The adsorption of ABH3 and His-ABH3 was evaluated in a bare capillary by using the pressure propagation technique described above (Fig. 1). When using buffer at pH 8.3, ABH3 (p*I* = 8.48) showed significant adsorption to the capillary surface. As shown in Fig. 1A, only 52% eluted towards the detector. Since the pH of the run buffer is marginally below the p*I* value of the protein, ABH3 is expected to have a net positive or near-neutral charge. Under these conditions, the positively charged ABH3 can interact with the capillary wall due to electrostatic attraction. The neutral ABH3 can undergo adsorption by orienting its surface towards the negatively charged wall to minimize charge repulsion and maximize charge attraction [35].

Under the same propagation conditions, His-ABH3 showed minimal adsorption to the capillary surface. Unlike the non-tagged protein, 92% of His-ABH3 eluted towards the detector (Fig. 1B). Since His-ABH3 is identical in all aspects to ABH3, the difference in adsorption can only be attributed to the His tag.

MutS (theoretical p*I* = 5.45) shows 94% elution during the buffer propagation stage (Fig. 2**A**). This is expected because at pH 8.3, MutS will have a negative charge and will not be able to interact with the capillary surface electrostatically. His-MutS, on the other hand, only shows 37% elution under the same conditions (Fig. 2C). Similar to the ABH3/His-ABH3 model system, the difference in adsorption in the MutS/His-MutS model system can only be attributed to the affinity tag.

His-FTO (theoretical p*I* = 5.10) also shows significant adsorption to the capillary where nearly half of the total protein is lost due to protein–wall interactions (Fig. 2E). The adsorption of FTO could not be measured since the non-tagged protein is not available. However, since the p*I* of FTO is similar to that of MutS, one can infer that the untagged protein will display less adsorption than the tagged version.

As expected, DNA eluted with a symmetrical peak during pressure propagation and maintains a consistent baseline throughout the run (Fig. 2G). In addition, DNA did not show any elution in a strong base. DNA does not adsorb to the capillary surface because its negative charge is repulsed by the negatively charged silanol surface of the capillary.

3.3. Contrasting effects of His tags on protein adsorption

His tags are used in recombinant proteins under the assumption that they do not alter the native properties of the protein. However, as shown in Figs. 1 and 2A, C and E, His tags can alter the adsorptive properties of proteins on bare-silica capillaries compared to the non-tagged proteins. In the His-ABH3 model system, the His tag decreases protein adsorption while in the His-MutS model system, it increases protein adsorption. The contrasting effects can be due to how the His tag influences the tertiary structure of the protein. All experiments in Figs. 1 and 2 are performed at pH 8.3. At these conditions, the histidine groups in the tag will be neutral in charge (pK_a = 7.21). These groups can support dipoles and hydrogen bond

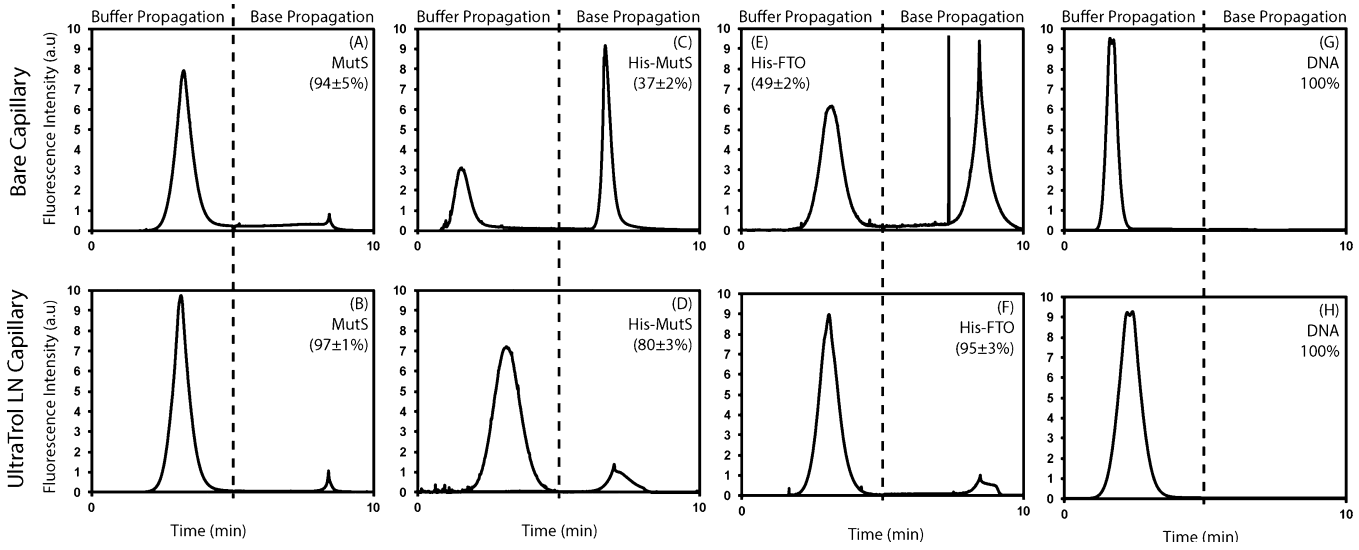

Fig. 2. Assessment of analyte adsorption onto the inner walls of a bare-silica capillary (top panels) and UltraTrol LN-coated capillary (bottom panels) for: 5 μM MutS (panels A and B), 2 μM His-MutS (panels C and D), 5 μM His-FTO (panels E and F), and 5 μM 80-nt long DNA library (panels G and H). Each analyte is pressure-propagated in 50 mM tris–acetate buffer first and in 1 M NaOH after that. The dotted line divides the two different propagation conditions and the percentage of analyte elution is indicated on the top corner of each electropherogram. Each experiment was performed in triplicate to calculate the average fraction of protein which elutes with buffer. Standard error of mean was used to determine statistical difference between each propagation condition.

Table 1
Average fractions of His-MutS which elutes with each buffer at different pH values in a bare capillary. Each experiment was performed in triplicate to calculate the average fraction of protein which elutes with buffer. Standard error of mean was used to determine statistical difference between each propagation condition.

50 mM Sodium phosphate pH 6.0	50 mM tris–Acetate pH 8.3	25 mM Borax pH 9.2
(1 ± 0.3)%	(37 ± 2)%	(34 ± 10)%

interactions which can alter the tertiary structure of a protein. If such interactions expose more positively charged groups on the protein surface, the protein may show increased adsorption. Inversely, if such changes expose more negatively charged groups to the surface, the protein may show decreased adsorption.

Changes in the tertiary structure of the protein can also influence apparent protein p*I*. The p*I* value of the protein is dependent on the pK_a value of each individual amino acid in its primary structure. The pK_a values of amino acids are influenced by their surrounding environment. If the tertiary structure of the protein changes, the environment for each amino acid may be altered. These changes can alter pK_a values of the amino acids, and subsequently, the p*I* of the protein. Alterations in the protein's p*I* can show adsorption patterns which deviate from the expected behaviour predicted from the theoretical p*I* of the protein.

3.4. Effect of pH on adsorption of His tag induced protein adsorption

A decrease in protein adsorption, as seen with the ABH3/His-ABH3 model system, is beneficial for KCE. An increase in protein adsorption, as seen with the MutS/His-MutS model system is detrimental for KCE. Since His tags are prevalent in most recombinant proteins, it is necessary to establish a preventative technique to reduce His-tag-mediated adsorption for KCE analysis. In all screening attempts mentioned below, His-MutS was used as a model system since it shows increased adsorption when compared to the untagged version.

In order to avoid adsorption, one of the easiest preventative techniques is the use of acidic (pH < 3.5) or basic buffers (pH > 10) to alter the charge of the analytes or capillary surface. The use of such buffers is not compatible with KCE because they can cause protein denaturation [10]. The charge of MutS and the His tag can still be manipulated by using KCE compatible buffers within a physiological pH range. As shown in Table 1, none of the KCE compatible buffers – 50 mM sodium phosphate (pH 6.0), 50 mM tris–acetate (pH 8.3) and 25 mM borax (pH 9.2) – are able to elute His-MutS at amounts comparable to MutS or to levels suitable for KCE analysis (greater than 80% elution). His-MutS adsorption persists even after increasing the pH above the p*I* of MutS and the His-tag. This likely indicates that the actual p*I* of His-MutS has increased when compared to the MutS protein due to alterations in the tertiary structure caused by the His tag. This observation casts doubt on the assumption that affinity tags do not influence protein structure [36].

3.5. Preventing His tag mediated protein adsorption by capillary surface coating

Another key technique used to prevent analyte adsorption is to alter properties of the silica capillary surface. All capillary coatings studied in our previous work, LPA (linear polyacrylamide), PVA (polyvinyl alcohol), and CElixer, were not successful at preventing His tag mediated adsorption of His-MutS (Fig. 3). LPA and PVA are permanent hydrophilic coatings which shield the negative charge of the capillary wall and, in many cases, inhibit protein adsorption. Since these coatings are permanently bonded, they cannot be washed with a strong base. To determine the degree of protein adsorption, His-MutS was injected into the capillary and pressure propagated in the buffer to determine peak symmetry. In a pressure-driven propagation test, His-MutS shows an asymmetric elution profile for both coatings. These profiles indicate protein adsorption onto the capillary wall (Fig. 3A and B). CElixer is a semi-permanent, commercial capillary coating which contains two components: a positively charged coating and a negatively charged buffer additive. His-MutS shows increased adsorption when using this coating where only 10% elutes towards the detector when propagated with 50 mM tris–acetate (pH 8.3). The increased adsorption may be due to the cationic coating component which allows the slightly negative His-MutS to interact with the capillary surface more than with a bare capillary (Fig. 3C).

The only coating which was able to reduce His tag mediated adsorption without causing DNA adsorption was UltraTrol LN, a novel KCE-compatible coating (Fig. 2B, D, F and H). UltraTrol LN is a commercial semi-permanent capillary coating utilizing N-linked polyacrylamide. For both His-tagged proteins, MutS and FTO, the UltraTrol LN-coated capillary improved peak symmetry and produced a consistent baseline throughout the run. In addition, the detection of adsorbed protein during base propagation showed that a much smaller fraction of His-MutS and His-FTO remain adsorbed to the capillary (Fig. 2B, D and F).

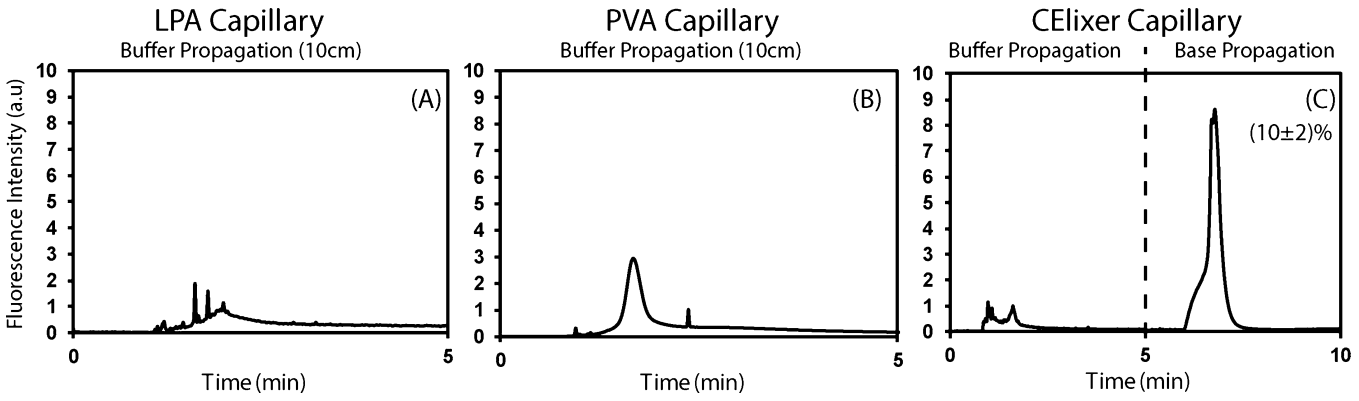

Fig. 3. Assessment of different KCE-compatible capillary coatings in preventing adsorption of 2.1 μM His-MutS: LPA permanent coating (A), PVA permanent coating (B), and CElixer dynamic coating (C). Propagation profiles in PVA- and LPA-coated capillaries were generated with a detector being 10 cm from the capillary inlet and the sample propagated by a pressure-induced flow in 50 mM tris–acetate (pH 8.3). The propagation profile in a CElixer-coated capillary was generated by using in 50 mM tris–acetate (pH 8.3) first and 1 M NaOH second. Experiments in the CElixer-coated capillary were performed with a detector 40 cm away from the inlet. The percentage His-MutS elution in CElixer capillary is indicated on the top corner of the electropherogram. The percentage and the standard error of mean were calculated as an average of three experiments.

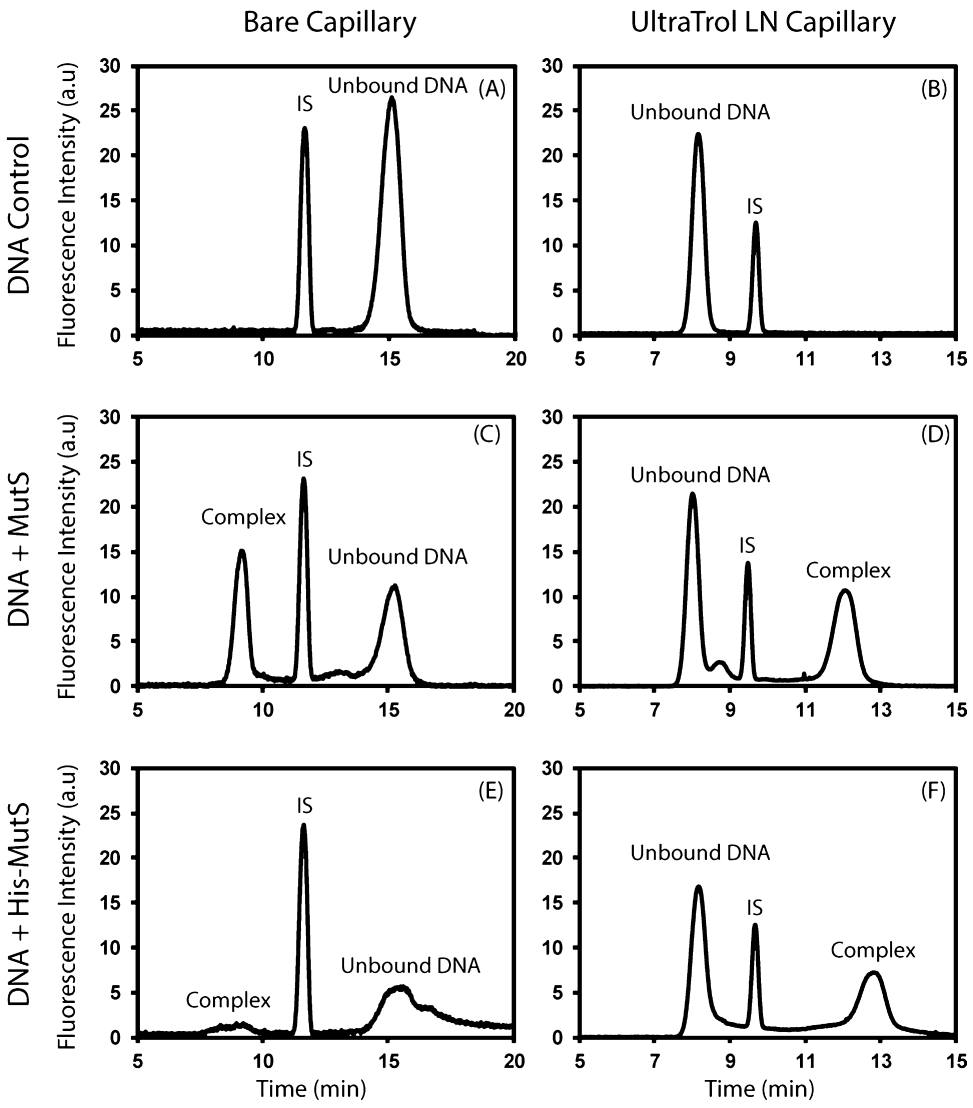

Fig. 4. NECEEM experiment performed with 100 nM His-MutS, 50 nM of MutS aptamer, and 10 nM internal standard (IS, fluorescein). (A) DNA in a bare capillary, (B) DNA in an UltraTrol LN-coated capillary, (C) DNA + MutS in a bare capillary, (D) DNA + MutS in UltraTrol LN capillary, (E) DNA + His-MutS in a bare capillary, and (F) DNA + His-MutS in UltraTrol LN-coated capillary. K_d was determined by comparing the area of unbound DNA with the area of DNA bound to protein using formula (2). The equilibrated mixture of His-MutS and aptamer was injected and propagated under reversed polarity at 600 kV/cm in an UltraTrol LN-coated capillary.

This observation suggests that UltraTrol LN inhibits adsorption of the two His-tagged proteins to the capillary walls. As seen by the symmetrical peaks and the lack of elution with a strong base, UltraTrol LN does not cause the adsorption of DNA (Fig. 2H).

When determining preventative techniques for protein adsorption, it is important to acknowledge that no surface can be completely free of protein adsorption. Proteins are complex macromolecules because they have heterogeneous surface charges which can change depending on protein conformation. Therefore, when selecting a preventative technique, one can at best choose a coating that targets the most prevalent conformation of the protein. In addition, the behaviour of an analyte within a capillary is highly dependent on the "history" of the capillary surface. If previous analytes in a capillary have altered the surface, the adsorption of the current sample can be either increased or decreased. Since the alteration of the capillary surface is a difficult-to-control process and conditioning a new capillary prior to each run is not feasible, we may expect at least a small amount of the protein to adsorb during the CE analysis.

3.6. *Influence of His tags on protein–DNA interactions*

Having established that UltraTrol LN is effective at inhibiting His-tag mediated adsorption, it is important to determine if the coating impacts KCE measurements. Semi-permanent capillary coatings can become unstable and elute with the run buffer. When the coating is introduced into the buffer, it may interact with the protein or DNA and thus interfere with protein–DNA interactions.

As shown in Fig. 4B, separations in an UltraTrol LN-coated capillary must be performed under a reversed polarity of the electric field. This is required because the EOF is directed from the cathode to the anode. Therefore, the elution profile for the complex, IS (internal standard) and unbound DNA is inversed compared to the profile generated in a bare capillary (Fig. 4A). As shown in Table 2, the K_d measurements for interactions involving MutS showed no statistically significant difference for the two capillaries. This suggests that UltraTrol LN does not interfere with biomolecular interactions and is thus KCE-compatible. As shown in Fig. 4E, the affinity of the MutS aptamer to His-MutS cannot be calculated in a bare capillary due to peak asymmetry caused by protein

Table 2
The K_d values for interaction of MutS and His-MutS with a previously selected MutS aptamer. The constants were calculated by using a protein titration experiment and NECEEM in a bare capillary and UltraTrol LN-coated capillary. Each experiment was performed in triplicate to calculate the average fraction of protein which elutes with buffer. Standard error of mean and a two-tailed *t*-test was used to determine statistical difference between each propagation condition ($p > 0.05$).

	Bare capillary NECEEM	UltraTrol LN Coated capillary NECEEM
MutS	(60 ± 4) nM	(70 ± 11) nM
His-MutS	Cannot be determined	(91 ± 12) nM

adsorption. The K_d value for His-MutS can only be calculated in a coated capillary, highlighting the importance of UltraTrol LN. A comparison of the K_d values for the non-tagged and tagged proteins in an UltraTrol LN-coated capillary shows that the His tag does not have a significant impact on aptamer affinity to the protein (Table 2). The latter observation supports the prevailing notion that affinity tags do not typically influence protein–DNA interactions.

4. Conclusion

The adsorption of analytes, particularly proteins, onto bare capillary surfaces limit the use of KCE. The inclusion of a His-tag can increase or decrease its adsorption compared to the non-tagged counterpart. Since His tags are used in many recombinant proteins, it is necessary to establish a technique to prevent His-tag mediated adsorption. In order to address His tag mediated analyte adsorption, this study investigated KCE-compatible coatings using His-MutS and His-FTO as model systems. We found that UltraTrol LN was the most effective at preventing the adsorption of His-tagged proteins. UltraTrol LN was also stable at physiological pH and did not interfere with biomolecular interactions, allowing for its use in the KCE analysis of His-tagged proteins.

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