Using Nonequilibrium Capillary Electrophoresis of Equilibrium Mixtures for the Determination of Temperature in Capillary Electrophoresis

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Until now, all methods for temperature sensing in capillary electrophoresis (CE) relied on molecular probes with temperature-dependent spectral/optical properties. Here we introduce a nonspectroscopic approach to determining temperature in CE. It is based on measuring a temperature-dependent rate constant of complex dissociation by means of a kinetic CE method known as nonequilibrium capillary electrophoresis of equilibrium mixtures (NECEEM). Conceptually, a calibration curve of “the rate constant versus temperature” is built using NECEEM and a CE instrument with a reliable temperature control or, alternatively, a nonelectrophoretic method, such as surface plasmon resonance. The calibration curve is then used to find the temperature during CE in the same buffer (cooling efficiency, length and diameter of the capillary, electrical field, etc.). In this proof-of-principle work, we used the dissociation of a protein–DNA complex to demonstrate that the NECEEM-based temperature determination method allows for temperature determination in CE with a precision of 2 °C. Then, we applied the NECEEM-based temperature determination method to study heat dissipation efficiency in CE instruments with active and passive cooling of the capillary. The nonspectroscopic nature of the method makes it potentially applicable to nonspectroscopic detection schemes, e.g. electrochemical detection. A “kinetic probe” can be coloaded into the capillary along with a sample for in situ temperature measurements. Higher order chemical reactions can also be used for temperature sensing, providing a kinetic CE method for measuring a corresponding rate constant is available.

Capillary electrophoresis (CE) is rapidly becoming an indispensable tool in studies of biomolecular interactions, such as protein–protein, protein–DNA, protein–small molecule, and DNA–small molecule.1–12 These affinity interactions are highly sensitive to temperature; therefore, they may be affected by Joule heat generated during CE. The reliable use of CE in such studies requires a means of accurately measuring the temperature inside the capillaries or channels during the course of electrophoresis. The use of temperature-sensing devices for measuring the temperature inside capillaries and channels during electrophoresis is technologically challenging. It is much more practical to use liquid probes, which can be sampled in CE without influencing the parameters of CE and the temperature of the separation media. Until now, all approaches for temperature sensing in CE relied on molecular probes with temperature-dependent spectral/optical properties. Temperature in capillaries and channels was measured with a number of spectroscopic techniques including NMR spectroscopy of water,13 backscattering of light,14 Raman spectroscopy of hydrogen bonds,15 light absorption by thermochromic liquid crystals and nanocrystals,16,17 and fluorescence spectroscopy of molecular fluorophores.18,19 The applicability of spectroscopic probes is obviously limited to specific spectroscopic detection schemes.

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phoresis of equilibrium mixtures (NECEEM).20–22 In this work, we used the dissociation of a protein–DNA complex to demonstrate that the new method allows for temperature determination in CE with a precision of 2 °C. We applied the new method to study heat dissipation efficiency in CE instruments with active and passive cooling of the capillary. Our result indicates that even CE instruments with active heat dissipation from the capillary have to be tested for the quality of heat dissipation for every set of experimental conditions before being used in temperature-sensitive analyses. The results also suggest that, in general, CE instruments with passive heat exchange should not be used for temperature-sensitive analyses.

With a number of advantages over conventional spectroscopic approaches, NECEEM-based temperature determination will find practical applications in CE method development for temperature-sensitive analyses, such as hybridization and affinity assays. Moreover, this work provides the opportunity for using NECEEM for complete thermodynamic characterization of the formation and dissociation of biomolecular complexes.

EXPERIMENTAL SECTION

Chemicals and Materials. Single-stranded DNA binding protein from Escherichia coli (SSB) and buffer components were obtained from Sigma-Aldrich (Oakville, ON, Canada). Fluorescently labeled 15-mer DNA oligonucleotides, fluorescein-5’-GCGGAGCGTGCCAGG, was kindly donated by Dr. Yingfu Li (McMaster University, Hamilton, ON, Canada). Fused-silica capillaries were purchased from Polymicro (Phoenix, AZ). All solutions were made using Milli-Q quality deionized water and filtered through a 0.22-μm filter (Millipore, Nepean, ON).

NECEEM. Two CE instruments were used in this work: a commercial P/ACE MDQ apparatus (Beckman-Coulter) with thermostabilization of the capillary (the outer walls of the capillary were washed with a liquid heat exchanger maintained at a fixed temperature) and a custom-made CE apparatus without thermostabilization of the capillary (the capillary was exposed to the ambient air).21 Both instruments employed laser-induced fluorescence detection with a 488-nm line of an argon ion laser for fluorescence excitation.

Uncoated fused-silica capillaries were used with the following dimensions: 40 cm × 20 μm i.d. × 350 μm o.d. in the commercial instrument and 40 cm × 20 μm i.d. × 150 μm o.d. in the custom-made instrument. Electrophoresis was run with a positive electrode at the injection end and an electric field of 400 V/cm if not indicated otherwise. The run buffer for all NECEEM experiments was 25.0 mM sodium tetraborate at pH 9.4. The samples were injected into the capillary by a pressure pulse of 1 s × 9.1 kPa; the length of corresponding sample plug was 0.93 mm as was calculated using the Poiseuille equation. The capillary was rinsed with the run buffer solution for 2 min prior to each run. At the end of each run, the capillary was rinsed with 100 mM NaOH for 2 min, followed by a rinse with deionized water for 2 min.

Equilibrium Mixtures. NECEEM experiments were performed with three different equilibrium mixtures of SSB and DNA (five repeats for every mixture). The protein and DNA were mixed in the NECEEM run buffer to have the final concentration of DNA equal to 100 nM and final concentrations of the protein equal to 0.25, 0.5, and 1 μM. The mixtures were incubated at room temperature for 1 h to reach the equilibrium prior to the analysis.

The rate constant of complex dissociation was calculated from a single NECEEM electropherogram by two methods: by fitting the dissociation curve with a single-exponential function and through peak areas.20–22

RESULTS AND DISCUSSION

In this work, we exploited the dissociation kinetics of a noncovalent complex between a SSB and fluorescently labeled 15-mer single stranded DNA (fluorescein-5’-GCGGAGCGTGCCAGG-3’):

\[
\text{SSS-DNA} \rightarrow \text{SSB + DNA}
\]

The rate constant, \( k \), of complex dissociation depends on temperature, \( T \), according to the Arrhenius equation:

\[
k = Ae^{-Ea/RT}
\]

where \( A \) is a preexponential factor, \( E_a \) is the activation energy of the reaction, and \( R \) is the gas constant.

First, we used NECEEM and a reference CE instrument (P/ACE MDQ) with controlled temperature of the capillary to study the dependence of complex dissociation kinetics on temperature.

The instrument was equipped with a laser-induced fluorescence detector to monitor the fluorescently labeled DNA and its complex with SSB; free SSB was not detectable. The equilibrium mixture of SSB and DNA was injected into the thermostabilized capillary by pressure, and its components were separated under nonequilibrium conditions.

NECEEM electropherograms changed drastically with the temperature of the capillary (Figure 1). These changes reflected temperature dependencies of the electroosmotic flow (shortening of the migration time with increasing \( T \)) and the dissociation rate constant, \( k \) (decreasing peak area of the complex). The rate constants at different temperatures were calculated using a standard NECEEM approach described in detail elsewhere20–22 and illustrated in the Supporting Information. The dependence of \( k \) on \( T \) was exponential, confirming the conformational integrity of the protein and a satisfactory quality of temperature stabilization in the reference CE instrument within the studied range of temperatures (Figure 2). This dependence can be used as a calibration curve for measuring an unknown temperature during CE with any CE instrument under any conditions with the only requirement being that the electrophoresis run buffer be the same. It should be noted that “the same buffer” requirement is not unique for NECEEM-based temperature determination—spectroscopic probes are also sensitive to buffer conditions so spectroscopic methods require calibration in the same buffer and with the same CE instrument. The calibration curve for the NECEEM-based method can be built with another CE instrument (e.g., the one with active cooling) or even with a non-CE method, such as surface plasmon resonance (SPR). The precision of the
NECEEM-based method ranges from 1 to 3 °C, depending on the temperature region.

Second, we used the new temperature determination method to study the quality of Joule heat dissipation in the reference CE instrument under different electric fields used to drive electrophoresis. The temperature of the capillary in the reference instrument was controlled by placing the capillary in a liquid heat exchanger. Joule heat is inevitably generated during electrophoresis, and if it does not dissipate efficiently, the temperature in the capillary will be greater than the temperature of the heat exchanger. Increasing heat generation, e.g., by increasing the electric field, when its dissipation is inefficient would lead to a further increase of temperature inside the capillary despite the constant temperature of the heat exchanger.

To examine whether heat dissipation was efficient in the reference instrument, we measured the temperature in the capillary as a function of an electric field under constant temperature of the heat exchanger. The temperature did not change with the electric field rising from 50 to 600 V/cm but increased dramatically when the electric field grew over 600 V/cm, suggesting that heat dissipation became inefficient when the electric field exceeded 600 V/cm (Figure 3). This result indicates that even CE instruments with temperature control of the capillary have to be tested for the quality of heat dissipation for every set of experimental conditions before being used in temperature-sensitive analyses.

Third, we applied the NECEEM-based temperature determination method to study heat dissipation efficiency in a CE instrument with passive cooling of the capillary through heat exchange with ambient air. The conditions of NECEEM were identical to those used in Figure 1 except for a capillary, which had thinner walls (65 instead of 165 μm) to promote more efficient heat dissipation. We found that even with thinner walls the temperature in the capillary during CE was 35 (±2 °C when the ambient air temperature was 20 °C. This result suggests that, in general, CE instruments with passive heat exchange should not be used for temperature-sensitive analyses.

Finally, we outline major characteristics of the NECEEM-based method for temperature determination in CE. The rate constant of complex dissociation measured by NECEEM depends only on the temperature and the buffer composition. It does not depend on other CE conditions such as capillary dimensions, capillary coating, or electric field. Thus, the calibration curve, \( k \) versus \( T \), measured with any reference instrument under any separation conditions (e.g., the one presented in Figure 2) can be used to determine the temperature for any other CE instrument, provided the complex-forming system and the electrophoresis run buffer are the same. Moreover, the calibration curve built with a non-CE method, such as SPR, can equally be used. This makes NECEEM-based temperature measurements simple and reliable. All spectroscopic methods, in contrast, require that a calibration curve be built with a CE instrument in which the temperature is
to be determined. This is often difficult, especially for CE on a chip. Another advantage of the NECEEM-based temperature determination is that, in contrast to spectroscopic methods, it can be performed with nonspectroscopic detection (e.g., electrochemical detection) provided a suitable complex is found. In addition, NECEEM provides much more reliable temperature information than spectroscopic methods, when used in CE with single-point detection (the majority of CE instruments employ this type of detection). NECEEM integrates temperature along the capillary length, while spectroscopic methods measure temperature in the detection point only. The temperature in the detection point may be considerably different from that in the rest of the capillary due to heating by interrogating light or difficulties with cooling in this point. As a result, spectroscopic methods may lead to significant systematic errors, while NECEEM should provide much more accurate temperature data. We would like to emphasize that a “kinetic probe” (e.g., DNA–SSB mixture) can be loaded into a capillary along with a sample for in situ temperature determination. To realize this mode, the sample must not interact with the components of the “kinetic probe” and a means should be provided for separate detection of the sample and the “kinetic probe” (e.g., by using different fluorescent dyes to label the sample and the detectable component of the “kinetic probe”).

To conclude, the NECEEM-based method represents the first nonspectroscopic technique for measuring temperature in CE. With a number of advantages over conventional spectroscopic approaches, it will find practical applications in CE method development for temperature-sensitive analyses, such as hybridization and affinity assays. Moreover, this work opens the opportunity for using NECEEM for complete thermodynamic characterization of the formation and dissociation of biomolecular complexes.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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