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Chemical Engineering Journal 135S (2008) S288–S291

Chemical Engineering Journal

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Direct circular dichroism spectra measurement of stretching long-strand DNA in a tapering microchannel

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Abstract

This paper reports the direct microchannel circular dichroism (CD) measurement method for analyzing conformational changes and orientations of macromolecules in a microchannel flow. CD is a sensitive probe for secondary structure. For that reason, we apply direct CD measurement for microchannels. Herein, we conducted the direct microchannel CD measurement method by devising an optical system in the sample chamber. Furthermore, using this CD microchannel measurement method, we studied conformational changes and orientations of long-strand DNA in an elongational flow.

Keywords: Microchannel; Laminar flow; Microfluidics; Orientation; DNA

1. Introduction

Macromolecule dynamics are of central interest in chemistry and biochemistry [1]. Conformations of polymer molecules, such as DNA strands, are typically studied in various kinds of flow [1–10]. It has been confirmed both theoretically and experimentally that DNA strands, which form a coiled state in a bulk solution, stretch or form various shapes in shear [1,2], elongational [4–8] and laminar flows [9,10]. In particular, elongational flow provides comparatively simple and complete stretching of polymer molecules. In addition to these reports, conformational control of macromolecules in a flow state has been studied. One example is efficient hybridization by stretching DNA strands [8–10].

Moreover, aside from DNA, it has been reported that the reaction rate of some enzymes using microreactors is higher than that for bulk scale enzymatic reactions [11–13]. However, the acceleration mechanism remains unclear. Similarly to studies of DNA, theoretical studies of conformational changes in fluid are applicable to most macromolecules, including enzymes and proteins [1–10]. Circular dichroism (CD) is a sensitive probe for secondary structure. Henceforth, we address direct CD measurement to elucidate conformational changes of macromolecules in a microchannel flow. Microchannel CD measurement, however, must confront difficulties in measurement because of the lack of light. Herein, we conducted the direct microchannel CD measurement method by devising an optical system in the sample chamber. Furthermore, using this CD microchannel measurement method, we studied conformational changes and orientations of long-strand DNA in an elongational flow.

2. Experimental

2.1. Microchannel-type flow cell

Fig. 1 shows the microchannels' designed and developed schematic composition, procedures, and velocity distributions. Fluid in a tapering microchannel follows an elongational flow. In a reverse-tapering microchannel, it follows a shortened flow. In the resultant velocity-gradient flow field, polymer molecules such as DNA are deformed by different directional strain rates. In the elongational flow, the extensional strain rates are introduced to stretch polymer molecules. DNA strands, which form a coiled state in a non-flowing (bulk) state, have been confirmed both theoretically and experimentally to stretch and orient themselves in an elongational flow [3–8]. In contrast, such stretching

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doi:10.1016/j.cej.2007.07.062

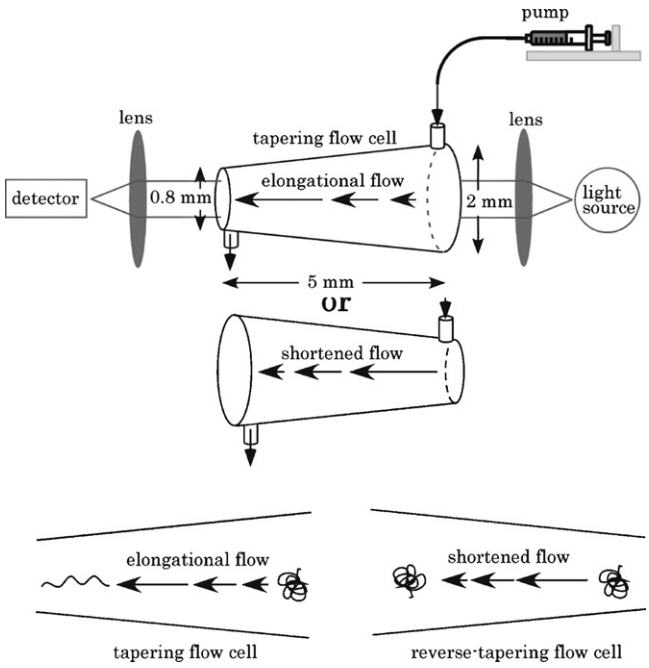

Fig. 1. Schematic showing composition, procedure, velocity distributions and DNA molecule behavior in tapering microchannels, along with component parts and arrangement in the sample chamber of the spectropolarimeter.

and orientation hardly occur in shortened flow; consequently, the polymer molecule remains in a coiled state.

2.2. Apparatus, chemicals and conditions

We measured CD spectra using a spectropolarimeter (J-820; Jasco Inc., Japan). Fig. 1 shows the arrangement of two lenses in a sample chamber to obtain parallel-luminous flux. An intelligent pump (PU-2080i; Jasco Inc., Japan) controlled all injections of solutions into the tapering flow cell (optical pass length: 5 mm, volume 44 μl). All measurements were carried out at 23 °C. Two aqueous solutions were prepared: (0.1 mM phosphate buffer (pH 7.0) and 0.1 mM NaCl) and (1 mM phosphate buffer (pH 7.0) and 1 mM NaCl) of 58 μM (per base pair) T4 GT7 DNA (Nippon Gene Co. Ltd., Japan).

3. Results and discussions

Fig. 2 shows CD spectra of DNA in non-flow and flow states (flow rate: 1 and 30 ml/min). Fig. 2a and b depict CD spectra measured in the same solution condition (0.1 mM phosphate buffer and 0.1 mM NaCl). Fig. 2a presents CD spectra of DNA in an elongational flow whereas Fig. 2b gives the measurement for a shortened flow. Fig. 2a shows no change in CD spectra observed between 0 and 1 ml/min. A gradual change in the CD spectra was observed upon increasing the flow rate beyond 1 ml/min. In this study, we confirmed the change in CD spectra from 1 to 30 ml/min, however, such change became gradual with higher flow rate. In an elongational flow, DNA strands overcome the tendency to be in the coiled state; the DNA strands stretch beyond a specific flow rate [3–14]. Results presented in Fig. 2a agree with this phenomenon. In contrast,

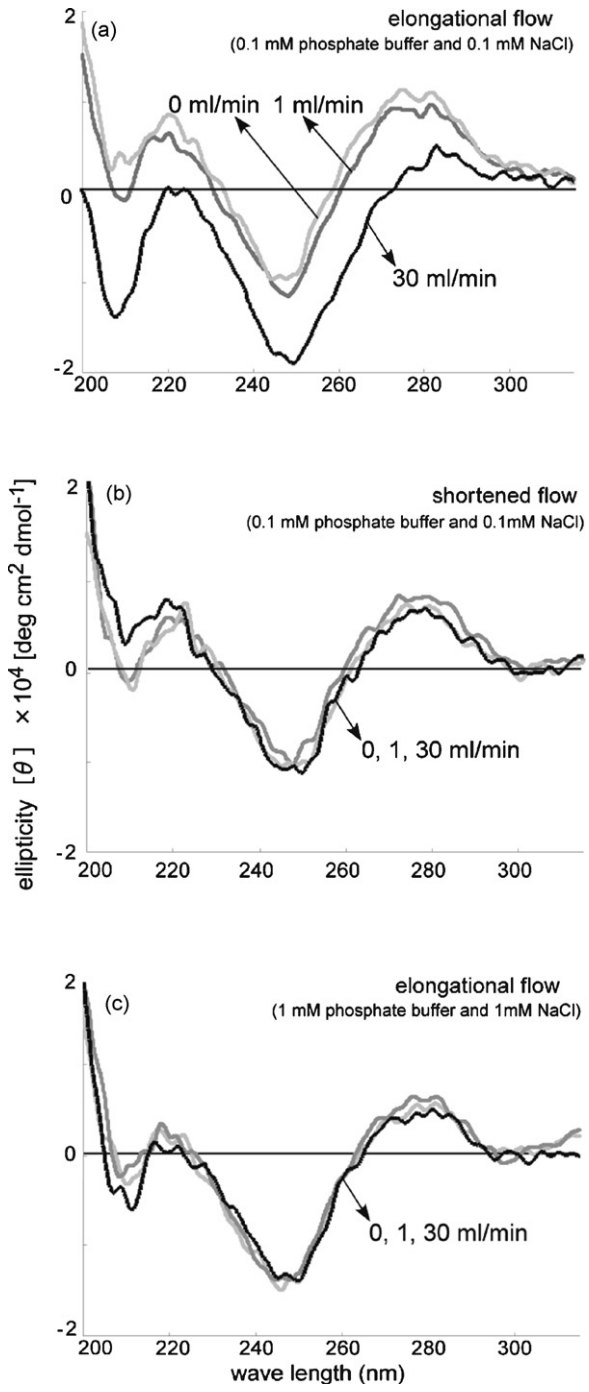

Fig. 2. Schematic CD spectra of T4 GT7 DNA solutions using a tapering microchannel flow cell under three conditions: (a) elongational flow, 0.1 mM phosphate buffer and 0.1 mM NaCl; (b) shortened flow, 0.1 mM phosphate buffer and 0.1 mM NaCl; (c) elongational flow, 1 mM phosphate buffer and 1 mM NaCl. Three kinds of flow speed were measured: 0 ml/min (non-flowing state), 1 and 30 ml/min.

no spectral changes were observed at any flow rate in a shortened flow (Fig. 2b): DNA strands do not stretch in a shortened flow.

On the other hand, a preparation of DNA in 1 mM phosphate buffer and 1 mM NaCl solution subjected to an elongational flow at different flow rates showed no change in CD spectra (Fig. 2c). This result indicates that DNA strands do not stretch in

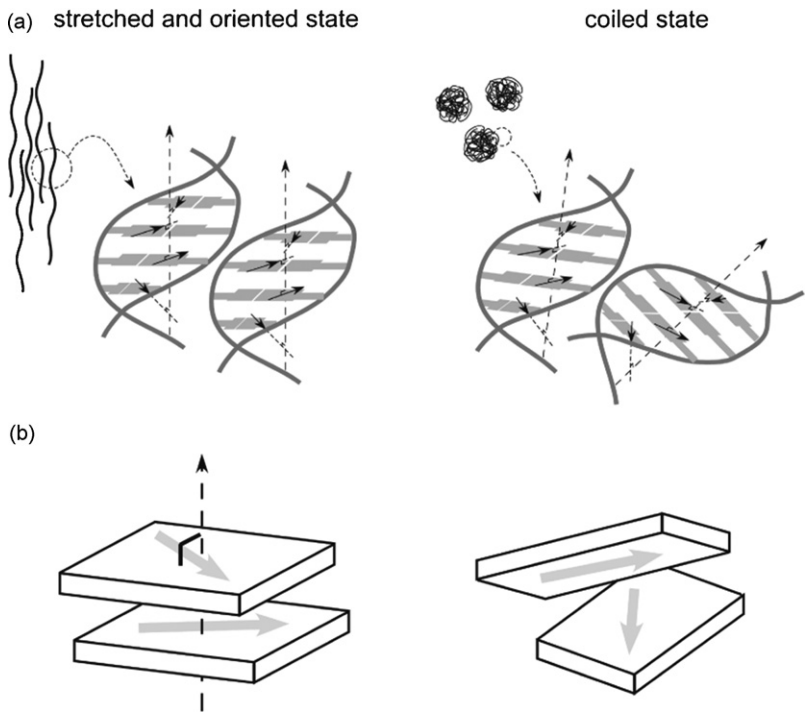

Fig. 3. Schematic explanation of CD spectra changes in elongational flow. (a) Electronic transitions of the bases are vertical to the helix axis at stretched and oriented states in elongational flow; in contrast, they are no discernible pattern in the coiled state. (b) At a stretched and oriented state, only those consistent in the helix-axis direction of electronic transition are not canceled out.

higher buffer and salt conditions. Stronger and smaller condensation of polyelectrolyte molecules, such as DNA, is engendered by higher salt concentration [15]. Our result in Fig. 2c shows agreement with this polymer behavior.

One of our experiments confirmed that changes in CD spectra occurred neither for chiral amino acid monomer nor in a buffer solution in microchannel elongational flow at flow rates of 0–30 ml/min (data not shown), implying that spectral changes result from the optical strain of the flow cell window that is related to a flow-rate increment. We also confirmed that no spectral changes occurred for any length of inner diameter tubes between the pump and flow cell, implying that the measured CD spectral changes reflect DNA conformational changes only in the flow cell.

Fig. 3 explains why changes in CD spectra occur upon stretching and orientation of DNA molecules. Phosphate groups in the DNA have electronic transitions only at wavelengths that are shorter than 170 nm. Deoxyribose shows low-intensity electronic absorption bands that begin at about 190 nm. Electronic transitions of DNA at 200–300 nm, the spectrum accessible region, are exclusively attributable to transitions of the planar purine and pyrimidine bases. All are polarized in the base plane, that is, vertical to the helix axis [16]. With coiled DNA in a non-flowing (bulk) state, the directions of all helix axes are no discernible pattern. In contrast, with stretched and oriented DNAs in an elongational flow, most helix axes are oriented in the flow direction (Fig. 3a). In this case, only those that are consistent in the helix axis direction of electronic transition are not cancelled (Fig. 3b). For that reason, the CD spectra change.

4. Conclusion

We have studied the direct microchannel CD measurement method and conformational changes and orientations of long-strand DNA in an elongational flow. Such microchannel CD measurement method might be useful for analyzing mechanisms of microchannel chemical reactions, particularly those reactions that involve conformation as an important factor for reactivity, such as enzymatic reactions.

Acknowledgements

This work was supported by the Special Coordination Funds for Promoting Science and Technology. This work was also partially supported by a Grant-in-Aid for Scientific Research (B) (No. 18310094) from the Ministry of Education, Culture, Sports, Science and Technology.

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