ANALYTICAL SCIENCES AUGUST 2000, VOL. 16

Separation of Red Blood Cells at the Single Cell Level by Capillary Zone Electrophoresis

Takao TSUDA,† Norihiro YAMAUCHI, and Shinya KITAGAWA

Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso, Showa, Nagoya 466–8555, Japan

By using capillary zone electrophoresis, the human red blood cell population was separated at the single cell level. A single injection of a human blood cell sample gave about one hundred peaks, and most of the peaks could be used to estimate the approximate number of cells. The separation of human red blood cells was also detected by a direct microscopic observation. The injection of a very diluted blood solution gave a few tens of peaks; and the peak heights were almost equal. The peak profile in the electropherogram corresponds directly to the distribution of the electrophoretic mobility of the red blood cells, which reflects on the cell properties, such as the surface charge density, volume and weight. Our finding is the first report to present the fine separation of population of red blood cells at the single cell level.

(Received January 6, 2000; Accepted May 12, 2000)

The outer walls of viruses and cells are generally charged. They can migrate electrophoretically under electric fields,[1–12] and the charge can be used for the manipulation of a single cell.[1,3,4] There are a few reports concerning the separation of viruses and cells. Most of the electropherograms of cells and viruses reported show a single peak,[5–7] although the peak might contain many cells with different properties. In other words, these separations only reflect the population of the cells (not the single cell).[1–4]

Because the surface of the human red blood cell (RBC) has negative charges that mainly come from sialic acid, the RBC migrates toward the positive electrode under an electric field. The migration velocity of the RBC depends on the properties of each cell, such as the surface charge density, volume and weight. The different electrophoretic mobility of blood cells might depend on the nature of the cell age and/or history of illness, such as diabetes mellitus. We have already reported on the relationship between the distribution of the electrophoretic mobility of diabetic RBCs and the hemoglobin A1c value.[9]

Capillary zone electrophoresis is a separation method based on the electrophoretic mobility of a solute. If it is possible to achieve the separation of cells at the single cell level, it could be possible to estimate the charge of the outer wall of the cell and to gain direct information concerning their characters. Such information may correlate with the state of health of a particular human being.

In the present study, we attempted to separate human blood cells in the single cell level by using CZE.

Experimental

Instrumentation

Two types of homemade apparatus for capillary electrophoresis were used in this experiment. One apparatus detected RBCs with visible light and the other apparatus was used for a direct observation. The former apparatus consisted of a fused-silica capillary (inner diameter: 50 µm), two reservoirs, a detector (on-column detection, CE-970, Jasco, Hachioji) and a high voltage power supply (Type HCZE-30NP, Matsusada Precision, Kusatsu). The reservoir was made of a 2.5 mL polyethylene syringe (Terumo, Tokyo), and a platinum wire was inserted as an electrode. In this homemade apparatus, the capillary column was set straightly without any bending.

Figure 1 shows the apparatus for the direct observation, which consisted of a microscope (BH-2, Olympus, Tokyo), a fused-silica capillary (inner diameter: 50 µm, effective length: 2.0 cm, whole length: 8.0 cm long), two polyethylene syringe reservoirs, a homemade high voltage power supply (0 to –10 kV), a CCD camera (CCD-X2, Shimadzu, Kyoto), and a video recorder-CRT monitor (VT-14M40, Sharp, Osaka). The homemade high voltage power supply consisted of a module of a high voltage power supply (HVR-10N, Matsusada Precision)

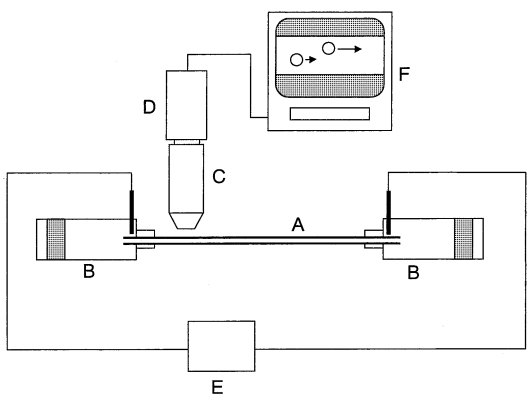

Fig. 1 Schematic diagram of an apparatus for direct observation. A: fused-silica capillary tubing; B: polyethylene syringe reservoir and platinum electrode; C: microscope; D: CCD camera; E: high voltage power supply; F: CRT monitor and video recorder.

† To whom correspondence should be addressed.
E-mail: takao@ach.nitech.ac.jp

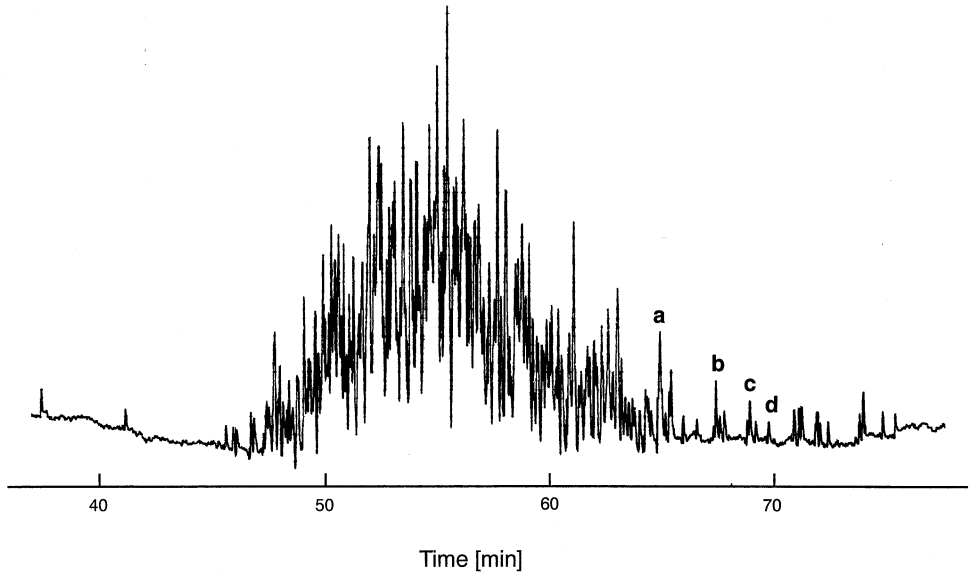

Fig. 2 Electropherogram of human red blood cells. Capillary: i.d. 50 μm, effective length 14.5 cm, whole length 25.2 cm; medium: saline containing 1% bovine serum albumin; applied voltage; 3 kV (130 V/cm); siphoning injection: 10 mm × 3 s; detection: visible light of 430 nm.

and a homemade voltage controller.

Modification of inner wall of a fused-silica capillary

To avoid the adsorption of red blood cells on the inner wall of the capillary, bovine serum albumin (BSA, Wako Pure Chemical Industry, Osaka) was coated on it. The procedure adopted was as follows. The fused-silica capillary column was filled with a 1% albumin aqueous solution and kept for 10 min. The solution was then flushed away with a nitrogen gas stream. The capillary was continuously dried with the nitrogen gas stream in an oven, the temperature of which was programmed to increase at a rate of 4˚C per min from 30 to 130˚C. This BSA-coated capillary column could be used for the analysis of human blood cells for more than one week without any problem.

Procedure

For the preparation of the sample solution, the whole blood was diluted with saline and its dilution ratio was 10 to 50 times. Each separation condition is described in the Figure caption. In the presented experimental condition, RBCs could migrate toward the positive electrode against the electroosmotic flow. Therefore, the RBCs were injected at the end of the capillary of the negative electrode side.

Results and Discussion

Typical electropherogram of human red blood cells

An typical electropherogram of human red blood cells produced by CZE is shown in Fig. 2. Red blood cells were detected with visible light of 430 nm, which was in the dominant absorption band of hemoglobin. After the siphoning injection of red blood cells, they migrated to the positive electrode against the electroosmotic flow. The electropherogram of red blood cells obtained has a fine structure and shows about one hundred peaks. The counter migration of RBCs had advantage to separation. The apparent electrophoretic mobility of the RBC, μ_{app}, is given by

$$\mu_{app} = \mu_{eph} + \mu_{osm} \quad (1)$$

where μ_{eph} and μ_{osm} are the real electrophoretic mobility of RBC and the electroosmotic mobility, respectively. In the case of counter migration, the sign of μ_{eph} is opposite to that of μ_{osm}. The advantage of the counter migration was explained by the following equation:

$$\Delta t_{AB} = \frac{L}{\Delta E}\left(\frac{1}{\mu_A + \mu_{osm}} - \frac{1}{\mu_B + \mu_{osm}}\right) \quad (2)$$

where Δt_{AB}, L, ΔE, μ_A and μ_B are the difference in the elution time between RBC-A and RBC-B, effective length, potential gradient, the electrophoretic mobility of RBC-A and RBC-B, respectively. In the case of $\mu_A < \mu_B$, the magnitude of the Δt_{AB} value increases in the range $-\mu_A < \mu_{osm} < 0$ compared to the value at $\mu_{osm} = 0$.

Without a sample injection the baseline was very stable and no spike could be seen. The spikes appeared only when we injected a sample, and after the elution of the red blood cells the baseline leveled again. Therefore, these spike peaks might be given by red blood cells and the separation might depend on the cell property at the single cell level.

The field flow fractionation (FFF) is also a method which can separate macromolecules and particles under a flow.[13-15] Because of the principle of the separation, the flow in FFF has to be Poiseuille flow. Namely, the difference in the flow velocity in a channel is one of the indispensable factors for FFF. The flow of the separation medium in CZE is electroosmotic flow and its flow profile is plug like flow.[16-18] Therefore, the separation of RBCs shown in Fig. 2 is not affected by the separation mechanism of FFF.

Electropherogram obtained by a direct observation

To confirm that each spike peak in Fig. 2 was able to identify the RBC, a direct observation was used as the detector for capillary electrophoresis. The electropherogram shown in Fig. 3 was obtained by using the apparatus shown in Fig. 1. The *x*-axis and *y*-axis in Fig. 3 mean the time and numbers of RBCs

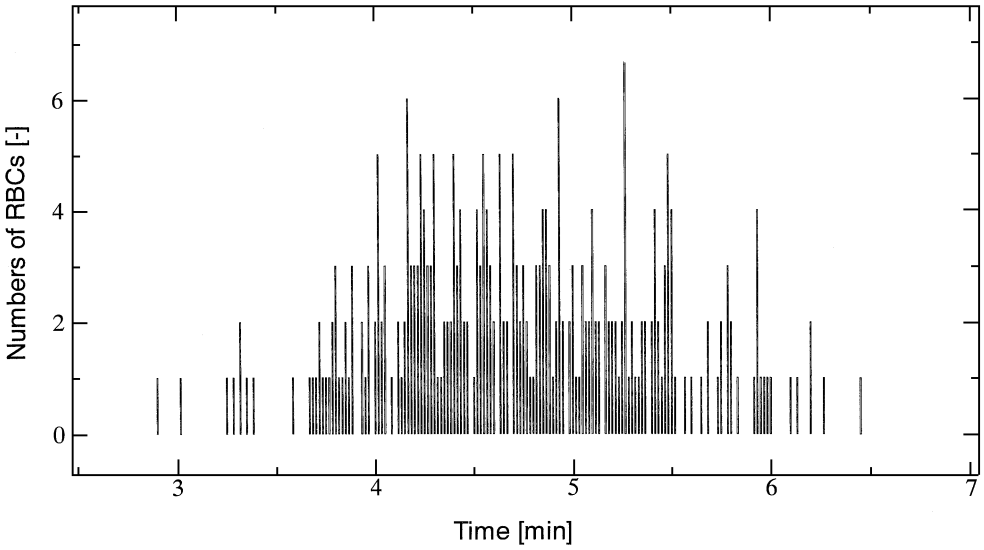

Fig. 3 Electropherogram of human red blood cells obtained by the direct observation *via* microscope. Capillary: i.d. 50 µm, effective length 2.0 cm, whole length 7.0 cm; medium: saline containing 10% glucose; applied voltage: 0.5 kV (71 V/cm); siphoning injection: 10 mm × 5 s; detection: microscopic reading.

counted by a direct observation. The procedure of counting by a direct observation was as follows. The migration of the RBC was observed with a magnification of *ca*. 1500 times on a CRT monitor. The number of RBCs that passed through a certain line with vertical direction on CRT during every second was counted.

The electropherogram in Fig. 3 was obtained by a microscopic observation at the single cell level. Therefore, the separation of human red blood cells at the single cell level was achieved by using capillary zone electrophoresis. Because the electropherogram in Fig. 3 was similar to Fig. 2, the spike peaks in Fig. 2 reflected the behavior of RBCs at the single cell level.

In Fig. 2, the separation of RBCs was performed at a migration distance of 14.5 cm; however, the separation of RBCs in short length was also attempted (Fig. 3). The 10% glucose was added to avoid the absorption of RBCs to an inner wall of the capillary. The addition of glucose gave the condition that the RBCs and the medium for CE were nearly equal in density. In this condition, the BSA was not necessary in the medium for CE to perform the separation of RBCs. The separation of RBCs in 2.0 cm long demonstrated the high performance of CE for cell (particle) separations.

Separation of RBCs at the single cell level

The electropherograms in Figs. 2 and 3 described that RBCs are able to separate at the single cell level by using capillary zone electrophoresis. These electropherograms correspond to the distribution of the electrophoretic mobility of RBCs. From a statistical point of view, when the number of RBCs injected in a capillary becomes small, the probability that two or more cells have nearly the same electrophoretic mobility also becomes small. Therefore, injection with a quite small sample volume would give separation at the single cell level. The peak heights in such a kind of electropherogram come to be nearly equal to each other because the adsorption of single RBCs can be regarded as a constant. The electropherogram shown in Fig. 4 was obtained by injection with a small sample volume. In Fig. 4, the detection wavelength used was changed from 430 nm to 550 nm because it gave a better signal-to-noise ratio (*S*/*N*). The

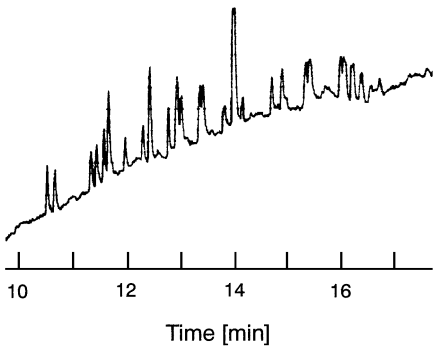

Fig. 4 Electropherogram of human red blood cells in single cell level. Capillary: i.d. 50 µm, effective length 7.7 cm, whole length 17.0 cm; medium: saline containing 1% bovine serum albumin and 0.025% agar; applied voltage: 3 kV (176 V/cm); siphoning injection: 5 mm × 5 s; detection: visible light of 550 nm.

0.025% agar was added into the medium for CE to increase the viscosity. Figure 4 shows a few tens of peaks, and their peak heights are almost constant. Namely, the peaks in Fig. 4 correspond to single RBC. A few large peaks might be given by the two or three RBCs.

The peak height corresponds to the number of RBCs. Therefore, it is possible to count the number of cells in each peak. The lowest height of the peak (**d** in Fig. 2) corresponds to a single cell. The peak of the double height of the lowest peak, **c** in Fig. 2, corresponds to two cells. Peaks **b** and **a** in Fig. 2 may correspond to three and six cells, respectively. Most of the peaks can be used to estimate the approximate number of cells included.

Conclusion

In the present study, the separation of human red blood cells at the single cell level was achieved by using capillary zone

electrophoresis. It is possible to estimate the approximate number of RBCs in each peak. The red blood cells were separated under three differential conditions. The electrophoretic mobility of each RBC might depend on the cell properties, such as the surface charge density, volume and weight, and the difference would give information about the human body.

Proof

The separation of red blood cells at the single cell level was first reported by us at the 72th Spring Meeting of Japan Chemical Society, Tokyo, March 27 – 30, 1997.

Acknowledgements

This research was partly supported by a Grant-in-Aid for scientific research from the Ministry of Education (No. 08650962) and Nakatani Electric Measuring Technology Association of Japan.

References

1. S. Kitagawa, C. Kawaura, O. Hashimoto, M. Naoi, and T. Tsuda, *Electrophoresis*, **1995**, *16*, 1364.
2. F. Foret, L. Krivanova, and P. Bocek, "*Capillary Zone Electrophoresis*", **1993**, VCH, Weinheim.
3. T. Matsue, N. Matsumoto, S. Koike, and I. Uchida, *Biochi. Biophys. Acta*, **1993**, *1157*, 332.
4. Y. Miwa, S. Yoshimura, and A. Itoh, *Int. J. Japan Soc. Prec. Eng.*, **1992**, *26*, 249.
5. S. Hjerten, K. Elenbring, F. Kilar, J. Liao, A. J. C. Chen, C. J. Siebert, and M. Zhu, *J. Chromatogr.*, **1987**, *403*, 47.
6. P. D. Grossman and D. S. Soane, *Anal. Chem.*, **1990**, *62*, 1592.
7. A. Zhu and Y. Chen, *J. Chromatogr.*, **1989**, *470*, 251.
8. S. Kitagawa, T. Tsuda, and O. Nozaki, *Bunseki Kagaku*, **1998**, *47*, 355.
9. S. Kitagawa, O. Nozaki, and T. Tsuda, *Electrophoresis*, **1999**, *20*, 2560.
10. P. C. H. Li and D. J. Harrison, *Anal. Chem.*, **1997**, *69*, 1564.
11. T. Tsuda, S. Kitagawa, and O. Nozaki, in Proceeding of the 10th International Symposium on HPCE, Kyoto, September **1997**, 67.
12. T. Tsuda, S. Kitagawa, and O. Nozaki, in Proceeding of the 72th Spring Meeting of Chemical Society of Japan, Tokyo, March **1997**, 104.
13. J. Janca, *Tr. Anal. Chem.*, **1983**, *2*, 278.
14. J. Janca, *Tr. Anal. Chem.*, **1987**, *6*, 147.
15. J. Yang, Y. Huang, X.-B. Wang, F. F. Becker, and P. R. C. Gascoyne, *Anal. Chem.*, **1999**, *71*, 911.
16. T. Tsuda, M. Ikedo, G. Jones, R. Dadoo, and R. N. Zare, *J. Chromatogr.*, **1993**, *632*, 201.
17. J. A. Taylor and E. S. Yeung, *Anal. Chem.*, **1993**, *65*, 2928.
18. T. Tsuda, S. Kitagawa, R. Dadoo, and R. N. Zare, *Bunseki Kagaku*, **1997**, *46*, 409.