A Microchip Capillary Electrophoretic Reactor: a New Methodology for Direct Measurement of Dissociation Kinetics of Metal Complexes

A microchip capillary electrophoretic reactor: a new methodology for direct measurement of dissociation kinetics of metal complexes†

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Received 5th July 2005, Accepted 29th July 2005
First published as an Advance Article on the web 8th August 2005
DOI: 10.1039/b509546h

A microchip capillary electrophoretic reactor has been proposed and successfully demonstrated in the direct evaluation of the solvolytic dissociation rate constant of the complex of Ce^{3+} with a polyaminocarboxylic ligand, 8-amino-2-[(2-amino-5-methylphenoxyl)methyl]-6-methoxyquinoline-*N*,*N*,*N*′,*N*′-tetraacetic acid.

The kinetic stability, *i.e.* inertness, is a key factor for the successful application of "functionalized metal complexes" such as "targeting metal complexes" for nuclear magnetic resonance diagnostic imaging. These complexes travel alone in human body for rather a long lifetime before excretion.[1] In such a situation, metal complexes are forced to be exposed to solvolytic dissociation reactions. Knowledge of the dissociation rate processes of metal complexes is necessary for design and development of analytical or diagnostic methods employing metal complexes under the environment in which free ligand and metal ions are separated from the vicinity of metal complexes.

In measuring the dissociation rate constants of metal complexes, some chemical methods relying on substitution reactions with proton, metal ion, and other ligands have been available. The direct measurement of the dissociation kinetics, however, has not been achieved using such conventional batch reaction techniques, because of difficulty in dividing between reactant (metal complex) and products (metal ion and free ligand). On the contrary, the capillary electrophoretic reactor (CER)[2] revolutionized the measurement of the dissociation rate constants of metal complexes since it involves direct measurements. The basic idea of a CER was provoked by the concentration jump character of the sharp band profiles in capillary electrophoresis (CE).[3] In CER, an electrophoretic buffer solution with no addition of ligand is employed.[4] Because of the differences in electrophoretic mobilities, during the CE separation processes the metal complexes move in their isolated bands along a capillary far apart from those of free ligands and free metal ions; free ligands and metal ions are separated from the vicinity of the metal complexes. Thus metal complexes are exposed to an overwhelming force to undergo a solvolytic dissociation reaction given as eqn (1) caused by a steep concentration jump of the ligand.

$$[ML] \rightarrow M + L \quad (1)$$

Solvent molecules are omitted, and ML, M, and L denote metal complex, metal ion, and free ligand. Then the solvolytic dissociation reaction of ML follows first-order kinetics. The solvolytic dissociation reaction starts immediately as electrophoresis is started and, consequently, the reaction time in CER is given as migration time of the metal complex. The decrement in the peak height absorbance of metal complex as the increment of reaction time and its first-order decay profile have been obtained by the accomplishment of several CE experiments with a variety of migration times. Thus the decay rate constant is given in the calculation of the decay curve.

Recently, miniaturized chemical separation devices have attracted great interest in producing rapid and reliable methods for chemical and biological analysis. Microchip capillary electrophoresis (MCE)[5] has the advantage of reducing separation time without reduction of separation efficiency when compared with conventional CE systems. From the standpoint of monitoring the dissociation kinetic processes in the time scale from several seconds to dozens of seconds, CER is less ideal, since CE separation with general CE apparatus requires minutes or longer to complete.

We report here the first attempt at utilizing MCE in the investigation of the dissociation kinetics of a metal complex. A microchip capillary electrophoretic reactor (μCER), is described providing a novel operative method for tracing the dissociation kinetic processes of a metal complex and directly evaluating its dissociation kinetics. The basic idea of μCER is constructed from that of CER. But, in contrast with CER, two attractive features of μCER, which arise from the employment of the MCE separation process as a chemical reactor for dissociation reactions of metal complexes in μCER, are demonstrated. First, the investigation for relativily fast dissociation kinetic process, which occurs in the time scale from several seconds to dozens of seconds, is performed. This is beyond CER. Second, UV absorption linear imaging detection[6] employed in μCER allows high-throughput data acquisition for the dissociation kinetic analysis. In μCER analysis, one can determine a dissociation rate constant of a metal complex with a single MCE analysis. By contrast, repetition of several CE experiments was necessary in CER analysis. The μCER system is successfully exemplified by evaluation of the dissociation rate constant of the Ce^{3+} complex with 8-amino-2-[(2-amino-5-methylphenoxyl)methyl]-6-methoxyquinoline-*N*,*N*,*N*′,*N*′-tetraacetic acid (Quin-2).

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† Electronic supplementary information (ESI) available: electropherograms extracted at intervals of 12 s from a series of electropherograms obtained successively in a single MCE separation run for the sample containing AS and DB (Fig. S1), and the values of plate numbers of AS and DB in various migration time (Table S1–S2). See http://dx.doi.org/10.1039/b509546h

Quin-2 in tetra potassium salt form purchased from Dojindo Lab. (Kumamoto, Japan) was dissolved in double distilled water (DDW). Ce^{3+} stock solution was prepared by dissolving the chloride salt in diluted hydrochloric acid (*ca.* 0.01M). 1,3-diaminobenezen (DB) and anthraquinone-β-sulfate (AS) were used as internal and external standard, respectively. All other reagents used were of guaranteed reagent grade.

A quartz glass microchip with a cross pattern channel (effective separation channel length: 25 mm) utilized in this study was obtained from Shimadzu, Corp. (Kyoto, Japan). The microchip had an elliptical channel cross-section of 50 μm wide and 20 μm deep. MCE separation and UV absorption linear imaging detection through the full length of the effective separation channel[6] was performed with a microchip electrophoresis system MCE-2010 (Shimadzu Corp.) at a detection wave length of 240 nm.

Before every run, a separation microchip is initialized by flushing a washing solution consisting of 0.1 M NaOH and 0.1 M sodium dodecylsulfate by pressure with a syringe, followed by rinsing thoroughly with DDW. The filling solution is then replaced with electrophoretic buffer (10 mM borate, pH 9.2). The sample containing 0.6 mM Quin-2, 0.6 mM Ce^{3+}, 10 mM borate and 3 mM DB is introduced electrodynamically in a separation channel with a pinched sample loading technique.[7,8] The stability constant of Ce^{3+}-Quin-2 complex ($[Ce^{III}L]^-$) is reported to be $10^{12.26\pm0.02}$,[9] and this is high enough to enable the formation of a stable 1 : 1 complex under the experimental conditions. The electrophoretic separation is started by applying a voltage of 60 V cm^{-1} and the separation time is 60 s. About 180 electropherograms are obtained successively in a single run as the sequential time-resolved UV absorption linear images of the effective separation channel taken every 0.3 s. Then, the electropherograms at intervals of 3 s are picked out and used for kinetic analysis. The migration times, t_m, and the peak height of $[Ce^{III}L]^-$ and DB are recorded for each of them. The same procedure is repeated for the sample containing 3 mM AS and 3 mM DB.

Fig. 1 shows the electropherograms extracted at intervals of 12 s from a series of 180 electropherograms obtained successively in a single MCE separation run for the sample containing $[Ce^{III}L]^-$ and DB. In every electropherogram, two peaks, $[Ce^{III}L]^-$ and DB, are present. There is no independent peak for free ligand, Quin-2, because of no addition of excess ligand to the sample. The internal standard, DB, is also utilized as a neutral marker. The rate of electroosmotic flow, μ_{eof}, can be estimated by calculation of the electrophoretic mobility of DB, because the neutral species are predominant at pH 9.2 for DB. The value of μ_{eof} was estimated to be $(7.58 \pm 0.19) \times 10^{-4}$ cm^2 s^{-1} V^{-1} from 5 electropherograms during a single experiment in Fig. 1. As the migration time is increased, both peaks move from anode (left) to cathode (right) and are separated from each other. It is noticed that the peak height of $[Ce^{III}L]^-$ decreases as the migration time increases. Of course, the peak height of "inert" DB is slightly reduced owing to the zone broadening due to longitudinal diffusion and other reasons.[10] The extent of the peak height reduction of $[Ce^{III}L]^-$, however, is much greater than that of DB. This shows that the concentration of $[Ce^{III}L]^-$ has been decreasing as it has been exposed to the solvolytic dissociation reaction during the electrophoretic migration separation process: the MCE separation process can work as a reactor for measurement of the dissociation reaction kinetics of a metal complex. The visualization of the dynamic behavior of the on-channel decomposition reaction of the metal complex is achieved with successive acquisitions of UV absorption linear images of the separation channel.

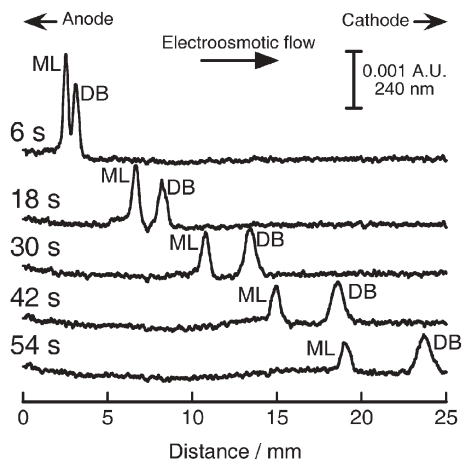

Fig. 1 Typical electropherograms obtained successively in a single MCE separation run for $[Ce^{III}L]^-$. Detection wavelength: 240 nm. Applied voltage: 60 V cm^{-1}. Separation time: 60 s. Electrophoretic buffer: 10 mM borate (pH 9.2). Sample: 0.6 mM Quin-2, 0.6 mM Ce^{3+}, 10 mM borate and 3 mM DB. ML: $[Ce^{III}L]^-$.

The solvolytic dissociation rate constant of $[Ce^{III}L]^-$ is derived from a series of electrophoretic data obtained during a single MCE analysis. The double standardization method[2] is employed for accurate measurement of the dissociation process of $[Ce^{III}L]^-$. The two types of standards employed, AS and DB, are both kinetically inert. DB is added as an internal standard for correcting the difference in injection volume between two samples, while an external standard, AS, which has an electrophoretic mobility value close to that of $[Ce^{III}L]^-$, is for estimation of the concentration of remaining $[Ce^{III}L]^-$ that survived during migration in the separation channel. For the kinetic analysis of $[Ce^{III}L]^-$, the peak height signals were employed because the peak area data may give unreliable results caused by asymmetric peak profiles owing to products of the dissociation reaction, such as free Quin-2.[2] The peak height signals of $[Ce^{III}L]^-$ and AS, normalized to that of DB in each electropherogram are H_{CeL} and H_{AS}, respectively. The basic idea of μCER is to measure the residue of the metal complex with increasing migration time, t_m, in order to obtain a first-order decay curve of dissociation. In this case, the residual ratio of $[Ce^{III}L]^-$ can be estimated using H_{CeL} and H_{AS},

$$[CeL^-]/[CeL^-]_0 = AH_{CeL}/HAS \quad (2)$$

where, $[CeL^-]_0$ is the initial concentration of $[Ce^{III}L]^-$ and A is a proportional constant.[11] Because the solvolytic dissociation reaction follows first-order kinetics, the rate law is given by,

$$d[CeL^-]/dt = k_d[CeL^-] \quad (3)$$

Integrating eqn (3) from $t = 0$ to t_m yields eqns (4) and (5).

$$\ln([CeL^-]/[CeL^-]_0) = -k_d t_m \quad (4)$$

$$[CeL^-]/[CeL^-]_0 = \exp(-k_d t_m) \quad (5)$$

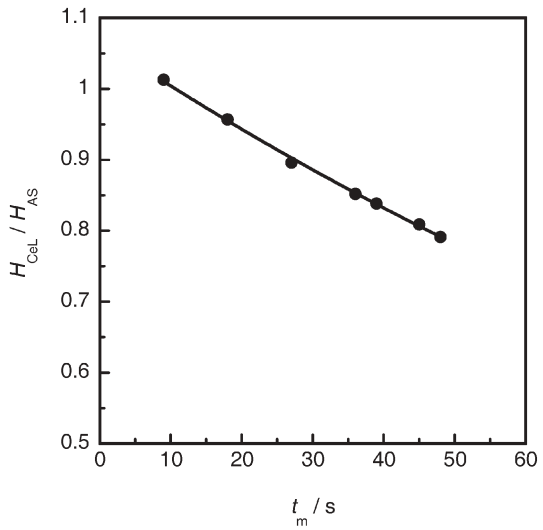

Fig. 2 Reaction profile obtained for $[Ce^{III}L]^-$ by μCER.

Eqn 6 is given by introducing eqn 2 into eqn 5.

$$H_{CeL}/H_{AS} = \alpha\exp(-k_d t_m) \quad (6)$$

Where, α is $1/A$. Here we can obtain k_d by fitting eqn (6) using the data H_{CeL}/H_{AS} at various t_m.

Fig. 2 shows the dependence of H_{CeL}/H_{AS} values on t_m. A simple first-order decay profile for $[Ce^{III}L]^-$ is observed, and plots are fitted well to eqn (6). The dissociation reaction rate constant for $[Ce^{III}L]^-$, k_d, is determined to be $(5.97 \pm 0.72) \times 10^{-3}$ s^{-1} from five experiments. The k_d value obtained with CER method,[12] $(7.04 \pm 0.51) \times 10^{-3}$ s^{-1}, is in good agreement with the above value. This proves the validity of kinetic analysis with μCER. In both μCER and CER, the peak height of $[Ce^{III}L]^-$ is normalized with respect to that of AS, therefore the effect of the difference in the mobilities of AS and $[Ce^{III}L]^-$ on the estimation of k_d is of concern.† In fact, k_d is overestimated when employing the single standardization method in which the residual ratio of $[Ce^{III}L]^-$ is estimated by normalization of the peak height signals of $[Ce^{III}L]^-$ with that of DB as inert internal standard, since apparent electrophoretic mobility of DB is greater than that of $[Ce^{III}L]^-$. The k_d value estimated indirectly using the ligand substitution reaction with EDTA is $(6.16 \pm 0.49) \times 10^{-3}$ s^{-1}[12] and is very similar to k_d obtained with μCER. Hence, this suggests that the effect of the difference in the mobilities of AS and $[Ce^{III}L]^-$ on the evaluation of k_d is very small. At the same time utilization of an external standard which is expected to have a close electrophoretic value to that of $[Ce^{III}L]^-$ is necessary in order to obtain a more accurate estimate. Thus, use of complexes of other lanthanide ions, such as Lu^{III}, with Quin-2 which are hardly dissociated in the time scale of MCE separation and consequently can be assumed to be inert as external standards, is now under investigation. It is stressed that one can acquire a set of data necessary for kinetic analysis during a single MCE run in this μCER system, while one must repeat several CE experiments with a variety of migration times to trace a decrease in the peak height absorbance of $[Ce^{III}L]^-$ and to obtain its decay curve in kinetic analysis with CER. Of course, it is much more simple compared to ordinary kinetic experiments with the requirement for complicated and tedious tasks.

In conclusion, we describe here the first application of μCER, a new high-throughput methodology for evaluation of the dissociation kinetics rate constant of metal complexes in the time scale from several seconds to dozens of seconds. Analysis of the determination of the residue of a metal complex within a single MCE separation process as a function of migration time allows the dissociation kinetics of the metal complex to be estimated rapidly. If the proper external standard materials are available, the μCER can actually extend not only to other metal complexes, but also to a wide range of complexes, so-called "molecular complexes" including biomolecular complexes, such as super-molecules, immuno-complexes, protein–protein or nucleic acid complexes, enzyme–substrate complexes, and so on.

Acknowledgements

This work was supported in part by Grant-in-Aid for Scientific Research (B), No. 15350039, from Ministry of Education, Culture, Sport, Science and Technology, Japan.

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