Notes

Dissociation Rate Constant Estimation for the Cerium(III)-*O,O′*-bis(2-aminoethyl)-ethyleneglycol-*N,N,N′,N′*-tetraacetate System by Capillary Electrophoresis

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The kinetic characteristics of lanthanide(III) chelates are very important to design a chemical system utilized for magnetic resonance imaging[1,2] and radioimmunotherapy.[3]   Such clinical applications have stimulated a number of dissociation kinetic studies on lanthanide(III)-polyaminocarboxylate chelates in solution.[4]   Generally, kinetic data can be obtained through a batch reaction system, such as a ligand or metal exchange. However, these measurements are useless in the case where the signal change accompanying these reactions is extremely small.

In this study, we featured capillary electrophoresis (CE) as a method for measuring the dissociation reaction rates to solve the problem mentioned above; this method has been applied for a kinetic investigation of the dissociation processes of Ce(III)-*O,O′*-bis(2-aminoethyl)ethyleneglycol-*N,N,N′,N′*-tetraacetate (egta) chelate.   The CE system used here is a kinetic differentiation mode capillary electrophoresis (KD-CE), which was reported in our previous papers.[5,6]   Since, in the KD-mode, no ligand ion is added in the carrier buffer solution, the injected chelates are spatially separated from the ligand to form an isolated band owing to their different mobilities.   Since there are no ligands and metal ion in the vicinity of the chelates moving on the electroosmotic flow (EOF), this brings about a very strong driving force for chelate dissociation, while the chelate migrates in a capillary to a detection window.   Thus, the chelates travel while being decomposed through the capillary. Time profiles of the dissociation processes are available by tracing the peak signal changes of the chelate as a function of the electrophoretic migration times.   In this study, various driving voltages, E, were imposed in order to control EOF, v_{eo}, based on the following relationship,[7] giving the changing migration times, *i.e.* the reaction times:

$$v_{eo} = -\varepsilon\varsigma E/\eta, \tag{1}$$

where ε, η and ς are the permittivity and viscosity coefficient of the carrier buffer, and zeta potential of the inner wall of a capillary.

An estimation of the proton-assisted and solvolytic dissociaton rate constants of the cerium(III) (Ce(III)) chelate with *O,O′*-bis(2-aminoethyl)ethyleneglycol-*N,N,N′,N′*-tetraacetic

acid (EGTA) was successfully achieved for the first time using the CE system.

Experimental

Chemicals and reagents

The reagent, EGTA (Dojindo Lab., Kumamoto, Japan), was dissolved in doubly distilled water and then neutralized to pH 7 with a minimum amount of sodium hydroxide.   Standard solutions of Ce(III) were prepared by dissolving its chloride salt (99.9% purity, Wako Pure Chemical Industries, Japan) in doubly distilled water with a few drops of concentrated hydrochloric acid.   The Ce(III) solution was standardized by EDTA titration with Xylenol Orange as an indicator.   The Ce(III)-egta chelate was prepared by mixing standard solutions of Ce(III) ion and the ligand solution.

Apparatus

The electropherograms were obtained with a CAPI-3200 electrophoresis equipment (Otsuka Electronics Co., Ltd., Japan).   On-column direct UV detection was carried out at 190 nm.   The temperature was set at 25°C.   A fused-silica capillary tube (0.05 mm i.d., total length: 42.2 cm, effective length to the detection window: 30 cm) was purchased from Scientific Glass Engineering Inc. (Australia).

Procedure

A solution of 2.0 mM Na_2HPO_4 (Kanto Chemicals, Japan) was employed as a carrier buffer at pH 6.5 – 8.   The sample solution (2.6 nl) was injected from the positive end by hydrodynamic action.   The high voltage from 1 kV (0.5 μA) to 20 kV (10 μA) was used to manipulate the apparent mobility of the chelate.   Jones and Grushka estimated that the temperature differences between capillary center and wall is 0.263 K when the electric power is 2 W (capillary length: 1 m, air temperature: 298 K).[8]   Since even the largest electric power is only 0.2 W in our case, the constancy of temperature in the capillary is likely to be secured at 298 K.   The dissociation rate law is deduced from the relationship between the peak heights and the migration times.   The peak heights were normalized with that of Bis-Tris as an inert internal standard.

Results and Discussion

A typical electropherogram is shown in Fig. 1.   The chelate,

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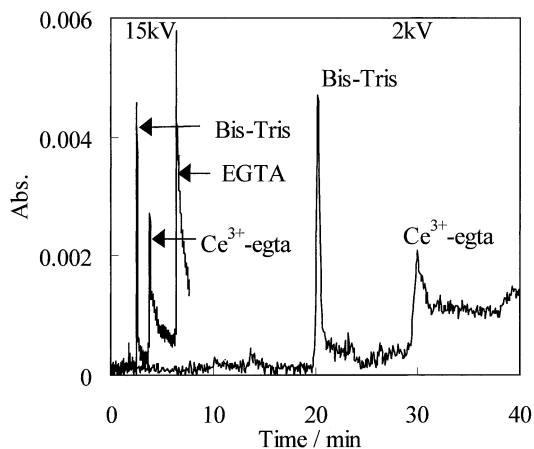
Fig. 1   Typical electropherogram for Ce(III)-egta chelate with different applied voltages.   Carrier buffer, $[Na_2HPO_4]_T$ = 2.0 mM; pH 7.0; capillary, 30/42.2 cm × 0.05 mm i.d.; applied voltage, 2 or 15 kV; sample, $[(Ce-egta)^-]$ = 1.0 mM, [Bis-Tris] = 10 mM; detection wavelength: 190 nm; temperature: 25°C.
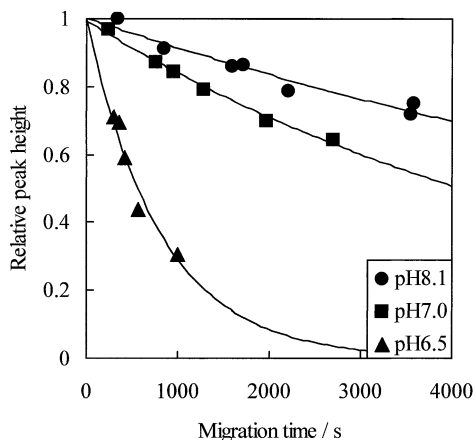

Fig. 2   Time courses of a dissociation reaction for Ce(III)-egta chelate in the CE system at different pH.   The solid line is a least-square-fitting curve.   Carrier buffer, $[Na_2HPO_4]_T$ = 2.0 mM; capillary, 30/42.2 cm × 0.05 mm i.d.; applied voltage, 1 – 20 kV; sample, [(Ce-egta)$^-$] = 1.0 mM, [Bis-Tris] = 10 mM; detection wavelength: 190 nm; temperature: 25°C.

$[Ce(III)$-egta$]^-$, was completely separated from the free ligand, $[H_2EGTA]^{2-}$, due to the difference of charge between the chelate and the ligand.   For a dissociation kinetic analysis of Ce-egta, the peak-height signals were exclusively used; the peak-area data are most likely inadequate because of the asymmetric peak profiles due to the slow moving product, H_2EGTA^{2-}.   It was observed that the peak height of the Ce(III) chelate decreased with increasing migration time, $i.e.$ the reaction time.   Since the peak heights of Bis-Tris with different applied voltages are almost the same as one another (Fig. 1), Bis-Tris is well suited as an internal standard.   The relationship between the peak heights and the migration times apparently corresponds to the first-order reaction decay curves (Fig. 2).   It should be noted that the CE separation of the chelate from the spectrally overlapping products, EGTA, gives an accurate time profile of the reaction.   The observed rate constant has a first-order dependence of the proton concentrations.   Therefore, the rate law is represented by the following equation:
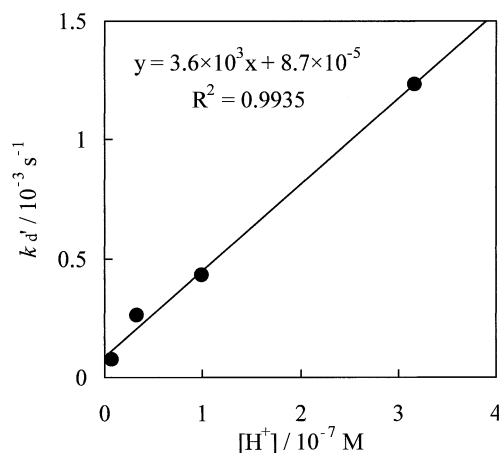

Fig. 3   Dependence of the observed rate constants (k_d') of Ce(III)-egta chelate on the proton concentrations.

$$-d[Ce\text{-}egta]/dt = k_d'[Ce\text{-}egta]$$
$$= k_H[H^+][Ce\text{-}egta] + k_d[Ce\text{-}egta]. \qquad (2)$$

The dissociation rate constants, k_d and k_H, for Ce(III)-egta chelate were determined from a linear least-squares fitting of the plots shown in Fig. 3.   The values of k_d and k_H for Ce(III)-egta chelate are $(8.7\pm4.5) \times 10^{-5}$ s^{-1} and $(3.6\pm0.2) \times 10^3$ s^{-1} M^{-1}, respectively.   These values are comparable with those for the Ce(III)-edta system (k_d = 2×10^{-4} s^{-1}, k_H = 2.9×10^3 s^{-1} M^{-1}).[9] The k_H for Ce(III)-cydta chelate, 60 s^{-1} M^{-1}, is much smaller than those for EGTA and EDTA chelates.   This is possibly due to the different rigidity in the bridge portion (cyclohexyl moiety) of the two iminodiacetate groups.   In order to validate these rate measurements by the CE method, the values of k_d' at pH 7 for Ce(III) chelates with 8-amino-2-[(2-amino-5-methylphenoxy)methyl]-6-methoxyquinoline-N,N,N',N'-tetraacetate   (Quin2)   and $trans$-1,2-diaminocyclohexane-N,N,N',N'-tetraacetate (CyDTA) were also measured in the same electrophoretic manner to that employed for the Ce(III)-egta system.   The k_d' values are $(1.1\pm1.1) \times 10^{-3}$ s^{-1} for Ce(III)-Quin2 and $(1.8\pm0.5) \times 10^{-4}$ s^{-1} for Ce(III)-cydta.   These values are almost the same as those measured through the ligand and metal-exchange processes in a batch reaction system $(4.0\pm0.5) \times 10^{-3}$ s^{-1} for Ce(III)-Quin2,[10] and 2.1×10^{-4} s^{-1} for Ce(III)-cydta chelate).[11]   The agreements prove the validity of the rate analysis by CE.

It should be emphasized that the batch reaction in solutions can not be used for investigating the Ce(III)-egta chelate, because (1) the absorbance change is so small on the ligand-exchange reaction to trace the time-course of the reaction, and (2) the proton-assisted dissociation path is predominant over the solvolysis one at the particular pH region where hydrolysis of the metal ion does not take place on the metal-exchange reaction.   In this case, a very small intercept of linear plots of $[H^+]$ $versus$ k_d' is given, from which the inaccurate solvolysis rate constant is derived.   However, the rate constant, k_d, can be readily measured with the CE method in a higher pH region.

In conclusion, the CE method serves as a facile and useful alternative for investigating kinetics in cases where signal disturbance and side reaction effects are significant.   The proposed CE method is useful to measure the rate constants, particularly for such chelates that give a small change in the absorbance upon decomposition and have a large k_H.

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