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Study of the racemization of L-serine by cyclodextrin-modified micellar electrokinetic chromatography and laser-induced fluorescence detection

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Abstract

Cyclodextrin-modified micellar electrokinetic chromatography and laser-induced fluorescence detection were used for the determination of the racemization rate of L-serine in water at 100°C. A difference to ten times the half-lifetime was observed between the value in the literature and the present results.

1. Introduction

Capillary electrophoresis (CE) is now well known for allowing separations of enantiomers by using the cyclodextrin-modified micellar electrokinetic chromatography [1]. The resolution of D- and L-amino acids has been studied by this means [2].

Because very small sample volumes are injected in CE studies, laser-induced fluorescence (LIF) is one of the most successful methods used to achieve high sensitivity and selectivity [3,4]. Many interesting analytes are not natively fluorescent and most reports on LIF detection with CE involve the use of visible lasers and labelling of analytes with flourescent functional groups, such fluorescein isothiocyanate for amines, amino acids or proteins [5].

A century ago it was first observed that amino acids underwent racemization when heated in strongly acidic and basic solutions. It soon became well established that the optically active amino acids isolated from biological materials could be converted into racemic mixtures by a variety of vigorous treatments. The earlier works on racemization in various systems was extensively reviewed in 1984 by Neuberger [6]. While racemization of amino acids at extreme pH and elevated temperatures has been known for a long time, it also takes place at neutral pH at rates which are comparable to those in dilute acids and bases [7].

Racemization was detected in fossils and the metabolically stable protein in living mammals [8], soils [9], trees [10] and wine [11] and was used in dating studies [12–14]. Kinetic data are not well established for a large number of amino acids [13,14].

Some years ago, Schwass and Finley [15] showed that serine is the most sensitive indicator of racemization among amino acids which can be measured, because of its particularly short

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racemization half-lifetime (around 400 years estimated at 25°C [13]). Kinetic studies are difficult because of the very small amount of enantiomer produced, owing to the very long reaction time.

To obtain new data on the racemization of serine, we used the high performance of separation by cyclodextrin-modified micellar electrokinetic chromatography and the high sensitivity of detection by LIF. We measured the kinetics of the reaction of a solution of a 10^{-8} *M* L-serine at 100°C in water. Differences from literature values were observed and are discussed.

2. Experimental

2.1. Apparatus and separation conditions

A modular injector and high-voltage power supply (SpectraPhoresis 100; TSP, Fremont, CA, USA) equipped with a modular CE–LIF detector (Zeta Technology, Toulouse-Ramonville, France) and a 488-nm wavelength laser (Type 54225A; ILT, Salt Lake City, UT, USA) were used. A 75 cm × 50 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used with an effective length of 42 cm.

All chemicals were purchased from Aldrich (St. Quentin Falavier, France).

The separation buffer consisted of 100 m*M* sodium dodecyl sulfate (SDS) (70% purity), 60 m*M* β-cyclodextrin and 100 m*M* boric acid (99.5% + purity) (pH 9.3, adjusted by addition of sodium hydroxide). The capillary was rinsed with 0.1 *M* NaOH for 3 min with water for 2 min and then with separation buffer for 3 min. Samples were injected by hydrodynamic injection for 2 s (15 nl). A separation potential of 28 kV was used. The electrophoretic current was typically 66 μA.

2.2. Data collection and analysis

Data collection, processing and analysis were performed using Boreal software (JMBS Developements, Grenoble, France). Data were collected at a sampling rate of 10 Hz. Peaks were identified by spiking samples with fluorescein isothicyanate (FITC)-labelled DL-serine. Average values for duplicate injections were calculated. The relative standard deviation ($n = 5$) of the peak height for 10^{-8} *M* DL-serine solution was 1.1%.

2.3. L-Serine kinetic study

Three solutions of 1.2 ml of 10^{-4} *M* L-serine (99% purity) in water (pH 7.8) were heated concurrently under reflux at 100°C. Volumes of 100 μl were taken after 2, 4, 6, 8, 22, 24, 26 and 55 h and derivatized with FITC at 20°C prior to CE–LIF analysis.

2.4. Derivatization procedure

A $2.1 \cdot 10^{-4}$ *M* solution of FITC isomer I (90% purity) in acetone was prepared by dissolving 2.5 mg of FITC in 3 ml of acetone. Then 2 mg of each amino acid were dissolved in 2 ml of 0.2 *M* carbonate buffer (pH 9.0). A 100-μl volume of serine solution (10^{-4} *M*) was allowed to react with 100 μl of FITC solution for 2 h in the dark. At the same time, 100 μl of a $2.1 \cdot 10^{-4}$ *M* solution of FITC in acetone was mixed with 100 μl of 0.2 *M* carbonate buffer to obtain a blank and kept in darkness for 2 h. Then both the FITC solution and the amino acid plus FITC solution were diluted 10 000-fold in water prior to analysis. D- and L-serine have the same kinetic reaction of labelling. The total concentration of serine was constant with time.

3. Results and discussion

Racemization is a well established first-order reaction and the kinetic equation for this reaction is [13]

$$\ln(1 + D/L)_t - \ln(1 + D/L)_{t=0} = K_i t \qquad (1)$$

where D/L is the L- to D-amino acid enantiomeric ratio at a particular time (t) and K_i is the first-order rate constant for the interconversion of amino acid enantiomer. This equation may be

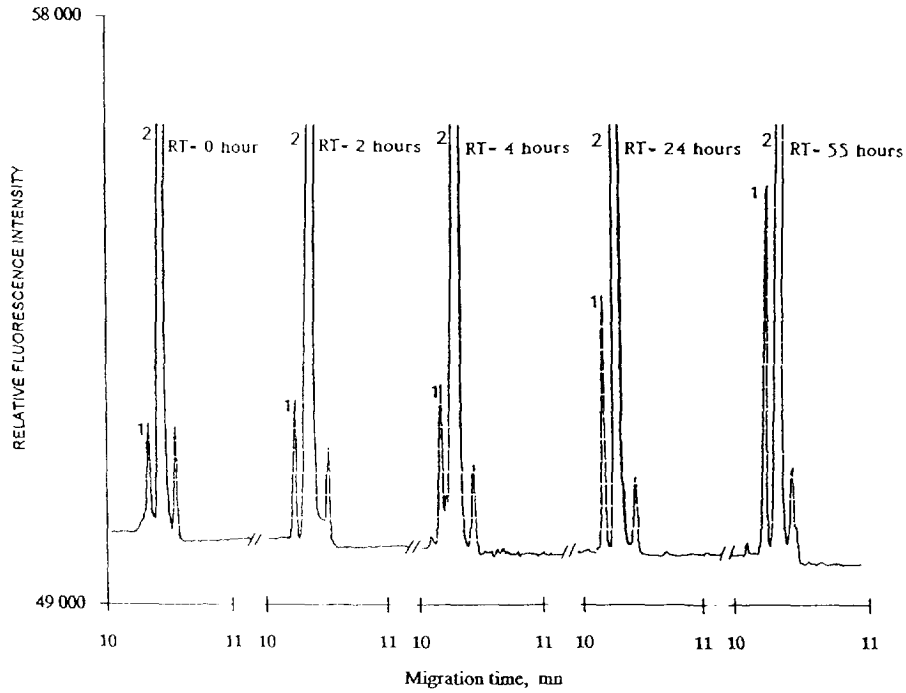

Fig. 1. Electropherogram showing 10^{-5} *M* FITC–L-serine racemization in water at 100°C after 0, 2, 4, 24 and 55 h of reaction. Separation conditions: SDS, 100 m*M*; β-cyclodextrin, 60 m*M*; boric acid, 100 m*M*; pH, 9.2; fused-silica capillary, 50 μm I.D., 28 kV, 66 μA. Peaks: 1 = D-serine; 2 = L-serine. RT = reaction time.

used when $D/L < 0.15$. In our case, for a reaction time of 55 h $D/L = 0.074$.

Fig. 1 shows five electropherograms of L-serine racemization after 0, 2, 4, 24 and 55 h of reaction. They show the growth of the peak of D-serine with time.

Mean values of the D/L ratio (R.S.D. < 4.49%) of the three reactions yielded a linear least-squares line fit:

$$\ln(1 + D/L) - \ln(1 + D/L)_{t=0} = 1.04 \cdot 10^{-3} t + 2.03 \cdot 10^{-3}$$

(t in hours) with a correlation coefficient of 0.9965 (the intercept was nearly zero).

The rate constant allowed the calculation of the racemization half-lifetime as 40 days for L-serine at 100°C at pH 7.8. The literature value [16] is 4 days, calculated from measurements at 122°C, assuming that the ratio of the rates of racemization of serine and aspartic acid are the same at the same temperature.

The present value shows that racemization of serine during protein hydrolysis will be relatively slow and will not have an important influence on the D/L ratio.

The results show that amino acids racemization may be observed with CE–LIF, which could be used as a dating method to determine the age of amino acid-containing samples.

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