ELSEVIER

Journal of Chromatography B, 741 (2000) 85–88

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Reaction rate, activation energy, and detection limit for the reaction of 5-furoylquinoline-3-carboxaldehyde with neurotransmitters in artificial cerebrospinal fluid

Jin Wu, Zhaohui Chen, Norman J. Dovichi*

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Abstract

The fluorogenic reagent 5-furoylquinoline-3-carboxaldehyde (FQ) has proven valuable in the analysis of proteins and small neurotransmitters. We monitored the reaction rate between this reagent and five neurotransmitters at 40° and 65°C in artificial cerebrospinal fluid. The reactions followed pseudo-first order kinetics. The activation energy for the reaction of FQ was 10.6, 10.7, 22.0, 31.4, and 34.4 kJ mol^{-1} for alanine, taurine, γ-aminobutyric acid (GABA), glutamine, and glutamic acid, respectively. At 65°C, the reaction rate was quite similar for alanine, taurine, glutamine, and glutamic acid (1.8×10^{-3} s^{-1}) but was twice as fast for GABA. A reaction time of nearly 1 h was required to quantitatively convert these neurotransmitters to their fluorescent products at 65°C. Detection limits for the labeled neurotransmitters were 10^{-9}–10^{-8} *M*, which corresponded to 0.3–7 amol injected onto the capillary.

Keywords: 5-Furoylquinoline-3-carboxaldehyde

1. Introduction

Capillary electrophoresis and laser-induced fluorescence have produced remarkable detection limits for highly fluorescent molecules. Single molecules of b-phycoerythrin have been detected by capillary electrophoresis with laser-induced fluorescence detection and yoctomole detection limits have been reported for fluorescently labeled DNA sequencing fragments, amino acids, and sugars [1–5].

While outstanding detection limits have been produced for fluorescent molecules, non-fluorescent analytes must be converted to fluorescent derivatives. Fluorogenic reagents have proven to be very valuable in the analysis of trace amounts of proteins and amines [6]. These reagents are non-fluorescent until they react with a primary amine, usually in the presence of a nucleophile. We are particularly interested in the reagent 5-furoylquinoline-3-carboxaldehyde (FQ). The fluorescent product generated by the reaction of FQ with a primary amine is excited by the 488-nm line of the argon ion laser. The emission has a large Stokes' shift, which makes it easy to spectrally filter the Raman scatter from the solvent. Proteins at extremely low concentration (10^{-13} *M*) have been labeled with the reagent and analyzed by capillary electrophoresis and laser-induced fluorescence [7,8].

In this paper, we consider the reaction rate and activation energy for the reaction between FQ and a set of neurotransmitters in artificial cerebrospinal fluid. This analyte system mimics both cerebrospinal

*Corresponding author. Tel.: +1-780-492-2845; fax: +1-780-492-8231.

E-mail address: norm.dovichi@ualberta.ca (N.J. Dovichi)

0378-4347/00/$ – see front matter
PII: S0378-4347(99)00540-X

fluid and brain dialysates. By studying the reaction in some detail, we are able to optimize the reaction conditions for rapid and high-sensitivity analysis of these compounds. Our goal is to minimize the sample manipulations, so the cerebrospinal fluid sample is analyzed without modification.

2. Experimental

2.1. Artificial cerebrospinal fluid

An alkaline stock solution was prepared by dissolving 23.6 g of NaCl, 9.2 g of Na_2CO_3, 1.2 g of KCl, and 0.32 g of Na_2HPO_4 in 100 ml of deionized water. An acidic stock solution was prepared by dissolving 2.4 g of $CaCl_2$ and 1.2 g of $MgCl_2 \cdot 6H_2O$ in 30 ml of concentrated HCl in a 45°C water bath. A glucose stock solution was prepared fresh daily by dissolving 0.8 g of glucose in 50 ml of deionized water. To make the working solution, 2.5 ml of the alkaline stock solution was diluted to 100 ml using deionized water. Next, 5 ml of the glucose stock solution was added. The pH was adjusted to 7.14 using the acidic stock solution.

2.2. Labeling reaction

A standard mixture of neurotransmitters was prepared in the artificial cerebrospinal fluid, with 3×10^{-4} *M* glutamine, 3×10^{-4} *M* glutamic acid, 1.2×10^{-4} *M* alanine, 6×10^{-5} *M* taurine, and 3×10^{-5} *M* γ-aminobutyric acid (GABA). A 2-μl aliquot of this standard neurotransmitter mixture was mixed with 4 μl of 25 m*M* KCN prepared in 10 m*M* borate buffer (pH 9.2) and with 100 nmol dry FQ reagent. The reaction mixtures were held in a thermostated chamber. Samples were diluted 100-fold with the artificial cerebrospinal fluid to quench the reaction. Rhodamine 6G was used as the internal standard. It was added to the diluted reaction mixture to make a 1×10^{-8} *M* solution.

2.3. Brain dialysate

The rat brain dialysate samples were kindly provided by Professor Glen B. Baker from the Neurochemical Research Unit, Department of Psychiatry, University of Alberta. A 4-μl dialysate sample and 4 μl of 25 m*M* KCN prepared in 10 m*M* borate buffer (pH 9.2) were mixed with 100 nmol of dry FQ reagent. The reaction was allowed to proceed in a water bath incubator (Fisher Scientific, Fair Lawn, NJ, USA) at 65°C for 50 min in the dark. Then 192 μl of artificial CSF was added to the mixture to quench the reaction. To correct for variations in injection volume, 2 μl of 10^{-6} *M* rhodamine 6G was added to the diluted mixture to make a 1×10^{-8} *M* solution.

2.4. Capillary electrophoresis conditions

A locally constructed capillary electrophoresis instrument with laser-induced fluorescence detection was used for this experiment [3]. Electrophoresis was performed with a 47-cm long, 15-μm inner diameter, and 145-μm outer diameter fused-silica capillary. Samples were injected at 5.64 kV for 3 s. Separation was performed at 18 800 V. The separation buffer was 15 m*M* borate, 45 m*M* SDS, and 5 m*M* β-cyclodextrin (pH 8.5). A 10-mW argon-ion laser beam at 488 nm was used for excitation. Emission was detected at 630 nm.

3. Results and discussion

Fig. 1 presents a typical kinetic curve obtained for GABA in artificial cerebrospinal fluid. The smooth curve is the least-squares fit of a first-order kinetic curve to the data

$$\text{signal} = \alpha(1 - e^{-kt}) \quad (1)$$

where α is a scale factor that is related to the sensitivity of the instrument, k is the reaction rate in s^{-1}, and t is reaction time in s. The precision of the data was improved by the addition of rhodamine 6G as an internal standard to the reaction products; this standard corrects for variations in injection volume and improves the precision by at least a factor of five. The reaction rate constant was also determined for GABA that was prepared in a mixture with alanine, taurine, glutamine, and glutamic acid; the rate constant for the reaction of GABA with FQ was identical to the value obtained from GABA alone.

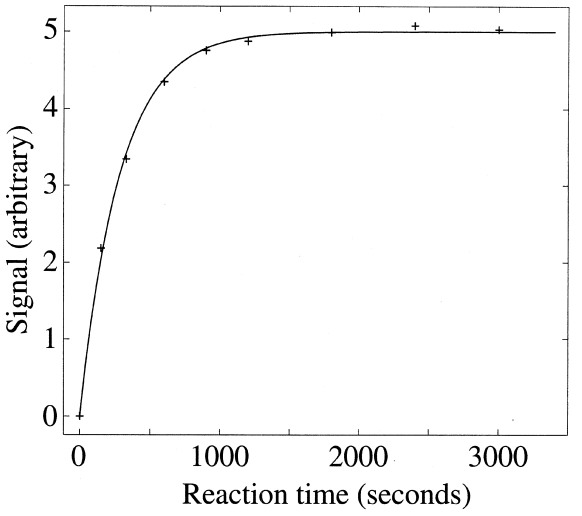

Fig. 1. Kinetic curve for the reaction of GABA with FQ in artificial cerebrospinal fluid at 65°C. The crosses represent the signal measured by capillary electrophoresis. The smooth curve is the least-squares fit of Eq. (1) to the data.

The reaction rate constant was determined for the other four neurotransmitters from the electrophoresis data generated on the mixture.

The reaction rate was determined at 40°C and 65°C, Table 1. The Arrhenius equation was used to estimate the activation energy for the reaction

$$k = A\exp(-E_a/RT) \quad (2)$$

where A is the pre-exponential term, E_a is the activation energy, R is the gas constant, and T is the absolute temperature. The activation energy is presented in Table 1 for the five neurotransmitters and values ranged from 11 to 34 kJ mol^{-1}.

Although the activation energy is relatively low for these reactions, the rate constant is not particularly high. The rate constant at 65°C is ~0.002 s^{-1} for most of the amines, although the reaction rate is roughly twice as fast for GABA. This sluggish reaction requires about 1 h to quantitatively convert the neurotransmitters to their fluorescent product, even at 65°C. This reaction rate is much slower than the labeling reaction of proteins by this reagent [8].

Concentration detection limits (3σ) were also determined for the fluorescently labeled amines (Table 1). A 10^{-4} M solution of amine was labeled and diluted to construct the calibration curve. Similar data were obtained by labeling a 10^{-6} M solution of amines. Detection limits ranged from 10^{-9} to 10^{-8} M. Concentration detection limits scale inversely with the capillary cross-section. The detection limit suffered from the use of a 15-μm I.D. separation capillary; the use of a 50-μm I.D. capillary would have improved the concentration detection limit by about an order of magnitude. The mass detection limit ranged from 300 zmol to 7 amol injected onto the capillary.

The best detection limit was obtained for GABA, which also had the fastest reaction rate of the five amines. Interestingly, the detection limit ranged by an order of magnitude for the other amines, even though they had a very similar reaction rate. This variation in detection limit does not reflect the kinetics of the reaction but instead may arise from differences in spectral properties of the product molecules, such as molar absorptivity, emission spectrum, and fluorescence quantum yield.

This labeling scheme is useful for the analysis of brain dialysates. Fig. 2 presents an electropherogram generated by labeling the dialysate with FQ and

Table 1
Kinetic data and detection limit for FQ labeled amines

Neurotransmitters	E_a (kJ mol^{-1})	Rate at 40°C (s^{-1})	Rate at 65°C (s^{-1})	LOD[a] (M)	LOD[b] (amol)
Alanine	10.6	0.00138	0.00187	2.4×10^{-9}	0.6
Taurine	10.7	0.00129	0.00175	2.3×10^{-9}	0.5
GABA	22.0	0.00179	0.00350	1.2×10^{-9}	0.3
Glutamine	31.4	0.00081	0.00198	14.0×10^{-9}	5.3
Glutamic acid	34.4	0.00071	0.00190	19.0×10^{-9}	6.7

[a] LOD = Concentration limit of detection (3σ) determined for the fluorescent products generated by a 3000 s reaction at 65°C.
[b] LOD = Mass limit of detection (3σ) for the fluorescent products generated by a 3000 s reaction at 65°C. This value is the product of the concentration detection limit and the injection volume.

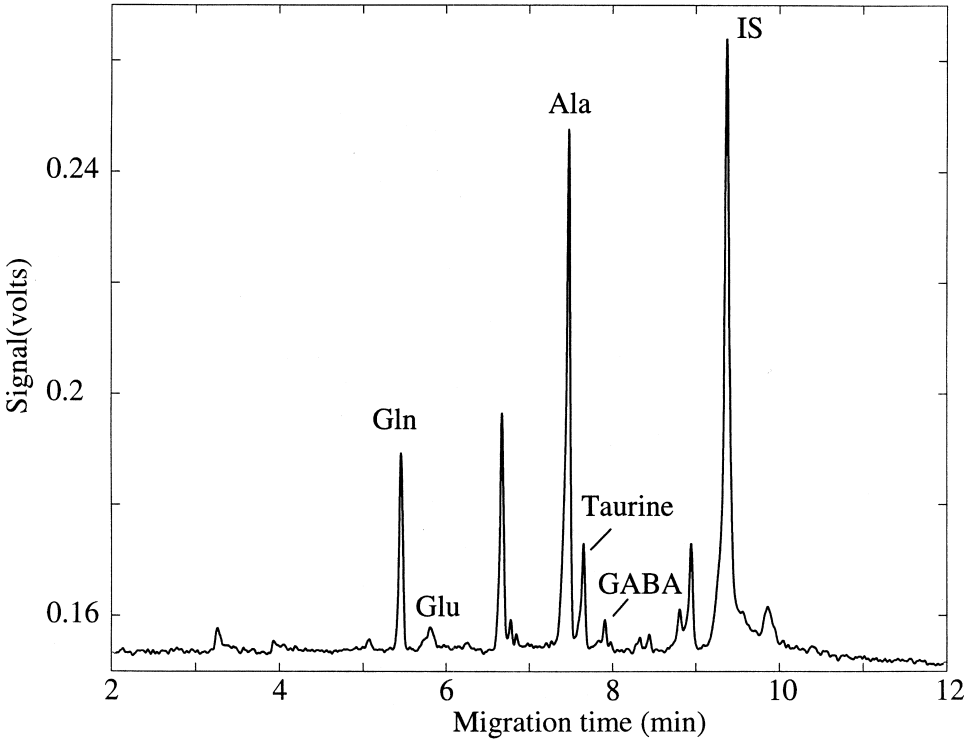

Fig. 2. Separation of fluorescently labeled rat brain dialysate.

spiking the sample with the internal standard before analysis. GABA is present in the sample and generates a peak that is slightly greater than the detection limit. Other amino acids are noted in the figure. Several unidentified peaks are also present, which may be due to other biogenic amines or small peptides that passed through the dialysis membrane.

Acknowledgements

This research was supported by an operating grant from the Natural Sciences and Engineering Research Council. We acknowledge the kind donation of the brain dialysate sample from Professor Glen Baker of the Neurochemical Research Unit, Department of Psychology, University of Alberta.

References

[1] D.Y. Chen, N.J. Dovichi, Anal. Chem. 68 (1996) 690–696.
[2] H. Swerdlow, J.Z. Zhang, D.Y. Chen, H.R. Harke, R. Grey, S. Wu, C. Fuller, N.J. Dovichi, Anal. Chem. 63 (1991) 2835–2841.
[3] S. Wu, N.J. Dovichi, J. Chromatogr. 480 (1989) 141–155.
[4] J.Y. Zhao, N.J. Dovichi, O. Hindsgaul, S. Gosselin, M.M. Palcic, Glycobiology 4 (1994) 239–242.
[5] J.Y. Zhao, P. Diedrich, Y. Zhang, O. Hindsgaul, N.J. Dovichi, J. Chromatogr. 657 (1994) 307–313.
[6] S.C. Beale, J.C. Savage, D. Wiesler, S.M. Wiestock, M. Novotny, Anal. Chem. 60 (1988) 1765–1769.
[7] D.M. Pinto, E.A. Arriaga, D. Craig, J. Angelova, N. Sharma, H. Ahmadzadeh, N.J. Dovichi, C.A. Boulet, Anal. Chem. 69 (1997) 3015–3021.
[8] I.H. Lee, D. Pinto, E.A. Arriaga, Z. Zhang, N.J. Dovichi, Anal. Chem. 70 (1998) 4546–4548.