ELSEVIER

Journal of Chromatography B, 695 (1997) 67–75

JOURNAL OF CHROMATOGRAPHY B

Identification of a recombinant synaptobrevin–thioredoxin fusion protein by capillary zone electrophoresis using laser-induced fluorescence detection[1]

Karen E. Asermely[a,*], Clarence A. Broomfield[b], Janet Nowakowski[c], Bernard C. Courtney[2,c], Michael Adler[a]

[a]*Neurotoxicology Branch, Pharmacology Division, U.S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5425, USA*

[b]*Biochemical Pharmacology Branch, Pharmacology Division, U.S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5425, USA*

[c]*Applied Pharmacology Branch, Pharmacology Division, U.S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5425, USA*

Abstract

Capillary zone electrophoresis (CZE) was utilized to identify a synaptobrevin–thioredoxin fusion protein (TSB-51). TSB-51 is a substrate for cleavage by botulinum toxin B at the Q(76)–F(77) site. TSB-51 was derivatized with a fluorophore, CBQCA [3-(4-carboxy-benzoyl)-2-quinoline-carboxaldehyde], for 4 h at room temperature. Optimal conditions for CZE separation of the TSB-51–CBQCA complex were determined: buffer (sodium borate), pH (9.0), applied voltage (25 kV), temperature (25°C) and forward polarity. SDS-PAGE showed that TSB-51 had a molecular mass of ~19 kDa. The protein was transferred to PVDF membrane and sequenced by the Edman degradation method verifying the first twelve amino acids as SDKIIHLTDDSF. TSB-51 was also collected during CZE separation and subsequently sequenced yielding the first three amino acids as SDK. This CZE–LIF method coupled with the CBQCA derivatization, fraction collection and Edman sequencing allowed for identification of the recombinant protein, a fast separation run time and utilization of small volumes of peptide (1.5 ng protein/23.6 nl injection). This method will be used for monitoring the endopeptidase activity of botulinum toxin B on TSB-51.

Keywords: Synaptobrevin; Thioredoxin; Botulinum toxin

1. Introduction

Botulism is a potentially fatal disease caused by one of seven serotypes of botulinum neurotoxin (BoNT) designated as A, B, C1, D, E, F or G [1]. It has been found recently that BoNT/B cleaves synaptobrevin II (also known as vesicle associated membrane protein II, VAMP II), leading to inhibition of acetylcholine release at the nerve terminal [2–4]. The clinical consequences of toxin-induced suppres-

*Corresponding author.

[1] Opinions or assertions herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or Department of Defense.

[2] Current Address: USAMRIID, Fort Detrick, Fredrick, MD 21701-5012, USA.

PII S0378-4347(97)00172-2

sion of transmitter release are paralysis of the diaphragm muscle, respiratory failure and death [1,2]. There is no known treatment to botulism following intoxication by BoNT/B or by other serotypes once a patient becomes symptomatic.

TSB-51 consists of 51 residues of synaptobrevin II (D44–K94) which includes the BoNT/B cleavage site (Q76–F77) coupled to the bacterial protein thioredoxin via an enterokinase cleavage site [5,6]. The advantage of studying TSB-51, instead of the entire 116 amino acid (aa) synaptobrevin II protein, is its enhanced solubility and ease of purification [6]. Synaptobrevin II and shorter fragments of synaptobrevin II have been identified previously by gel electrophoresis [3], high-performance liquid chromatography (HPLC) and mass spectrometry [7,8], Western blot analysis [9,10], immunoprecipitation [11,12] and nuclear magnetic resonance spectroscopy [13].

CZE was first described by Jorgenson and Lukacs [14] as a method of separation of dipeptides as fluorescamine derivatives in open-tubular glass capillaries. Since the original method was introduced, various CZE studies with different recombinant proteins and peptides have been published; detection limits of 10^{-18} mol are possible when peptides are derivatized with the fluorescent probe 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde, CBQCA [15].

In this study, CZE with laser induced fluorescence (LIF) detection was used to characterize TSB-51, and was preferred to other methods due to an ability to process smaller sample injection volumes (nanoliter range) and short separation run times [14,15]. Optimal conditions for the separation of TSB-51 by CZE–LIF were determined with respect to voltage, current, Joule heating, temperature, pH, buffer system, injection time and polarity. The molecular mass of the fusion protein was confirmed by SDS-PAGE gels. The identity of the TSB-51 was verified by polyacrylamide gel separation followed by gel blotting to PVDF membrane and Edman analysis of the first 12 amino acids [16]. Due to a small amount of TSB-51 obtained by fraction collection of the capillary effluent [17], only the first 3 amino acids were verified by Edman sequencing. CZE–LIF was found to be a highly reproducible and sensitive method and will be used in future studies to monitor the cleavage of TSB-51 by BoNT/B.

2. Experimental

2.1. Equipment

CZE experiments were performed on a Beckman PACE 2100 System with a 3 mW argon ion laser. Settings were adsorption wavelength 488 nm and emission wavelength 520 nm, with analysis done using System Gold computer software. An open uncoated silica capillary (57 cm×75 μm I.D.×375 μm O.D.) was used.

Milliblot Electroblotter System was obtained from Millipore Corp. (Bedford, MA, USA). Immobilon-psq PVDF membrane was obtained from Millipore Corp.

Protein sequencing was carried out on a Beckman LF 3000 Protein Sequencer (Beckman Instruments, Columbia, MD, USA).

LASERGENE computer software, 1994 (DNASTAR Inc., 1228 S. Park Street, Madison, WI 53715).

2.2. Chemicals

The vector system (pTrxFus™) used for cloning synaptobrevin–thioredoxin fusion protein was obtained from Invitrogen Corp. (San Diego, CA, USA). The DNA for the fragment of synaptobrevin II studied was obtained from Dr. John Middlebrook (USAMRIID, Ft. Detrick, Frederick, MD, USA).

The following reagents and sources were used: fluorescein (sodium salt) (Beckman), capillary regenerator solution A (Beckman), run buffer A (Beckman), lysyl-glycine HCl (Sigma Chem. Co., St. Louis, MO, USA), osmotic shock buffer #2 (Invitrogen Corp.), CBQCA (Molecular Probes, Eugene, OR, USA) and 10–20% gradient Tris–tricine SDS-PAGE gels, run buffers and molecular mass standards (BioRad Labs, Hercules, CA, USA). A standard mixture of 19 PTH-amino acids were obtained from Sigma. All chemicals for the protein sequencer were Beckman Grade.

2.3. Conditions

2.3.1. Sample preparation

DNA coding for the 51 amino acid fragment of synaptobrevin II was cloned into the pTrxFus vector and expressed in *E. coli* cells as a synaptobrevin–

thioredoxin fusion protein (TSB-51) using the Thiofusion Expression System™ (Invitrogen Corp.) [6]. The fusion protein was isolated by osmotic lysis in 2 ml buffer containing 20 m*M* Tris–HCl, 2.5 m*M* EDTA, pH 8.0. To generate a fluorescent derivative of TSB-51, aliquots of osmotic shock buffer containing 26–47 μg/ml TSB-51 were added to CBQCA (10 m*M*) and potassium cyanide (20 m*M*) in a volume ratio of 1:1:2, respectively. The final concentration of TSB-51 was 0.49 μ*M* [6]. The mixture was kept at room temperature for 4 h during derivatization [15]. The dipeptide marker, lysyl-glycine (30 n*M*), was added to the fusion protein and derivatized with CBQCA.

2.3.2. Capillary zone electrophoresis

Buffers. Pre-made buffers were obtained from Applied Biosystems (Foster City, CA, USA). These consisted of 40 m*M* sodium phosphate buffer, pH 2.5; 40 m*M* sodium acetate buffer, pH 4.0; 40 m*M* sodium phosphate buffer, pH 7.0; and 40 m*M* sodium borate buffer, pH 9.0. A stock solution of 400 m*M* Tris–HCl was made and adjusted to pH 8.0 with 1 *M* HCl, then diluted 1:10 for working buffers (40 m*M*).

Other conditions. The sample pressure injection time was 4 s except for fraction collection experiments in which 15 s injections were used.

2.4. Effect of voltage on the migration time of TSB-51

The fusion protein was derivatized with CBQCA. The protein was pressure injected for 4 s into an open uncoated silica capillary (57 cm×75 μm I.D.× 375 μm O.D.) and separated on the CZE using the run buffer sodium borate (40 m*M*). Different voltages were applied (10, 20 and 30 kV) for 25 min at a constant temperature of 25°C.

2.5. Effect of pH on the migration time of TSB-51

The fusion protein was derivatized with CBQCA. The protein was pressure injected for 4 s into an open uncoated silica capillary (57 cm×75 μm I.D.× 375 μm O.D.) and separated on the CZE using run buffers of different pH: 40 m*M* sodium phosphate, pH 2.5, 22.5 kV; 40 m*M* sodium acetate, pH 4.0, 30 kV; 40 m*M* sodium phosphate, pH 7.0, 17.5 kV; 40 m*M* Tris–HCl, pH 8.0, 27.5 kV; and 40 m*M* sodium borate, pH 9.0, 25 kV. The temperature was constant at 25°C.

2.6. Effect of temperature on the migration time of TSB-51

Prior to derivatization, the CE was set at 20, 25 or 30°C and allowed to equilibrate for 30 min. The fusion protein was derivatized with CBQCA. The protein was pressure injected for 4 s into an open uncoated silica capillary (57 cm×75 μm I.D.×375 μm O.D.) and separated on the CZE using a run buffer of 40 m*M* sodium borate, pH 9.0 with an applied voltage of 25 kV.

2.7. Time course of CBQCA labelling of TSB-51

TSB-51 was labelled with CBQCA. Measurement of the separation of TSB-51 started at 2 min. Every 60 min, a sample was injected into the CE and separated to monitor the amount of CBQCA labelling. Lysyl-glycine (30 n*M*) was used as a marker.

2.8. Fraction collection of TSB-51

TSB-51 was derivatized with CBQCA as described. The sample was separated on CE at 25°C, using 40 m*M* sodium borate buffer for 10 min. The major peak in the separation was collected in 2 μl of 40 m*M* sodium borate buffer (pH 9.0) in a microvial. Collection of TSB-51 was carried out for two 4-h periods, and the sample was pooled in the same microvial. The collected sample was frozen at −20°C until sequencing.

It was observed by Liu et al. [15] and in this study that CBQCA-derivatized proteins are not stable in acidic buffers. Taking advantage of this fact, the TSB-51–CBQCA complex, that was fraction collected (40 times and pooled) and frozen at −20°C, was thawed to room temperature and exposed to 1 *M* HCl for 1 h to remove the CBQCA from the N-

terminal primary amine group of TSB-51. Pooling of 40 collections of TSB-51 (obtained from 40 injections of 0.38 ng *E. coli* lysate/5.9 nl injection/s on the CZE) provided only enough protein for 3 cycles of sequencing.

2.9. Tris–tricine SDS-PAGE gels and blotting

The procedure used for Tris–tricine SDS-PAGE gels was that of Schagger and von Jagow [18]. *E. coli* lysate (750 ng) was loaded on a SDS Tris–tricine gel (10–20%) and run at constant voltage (30 V for 1 h then 60 V for 3 h). Blotting was performed according to the technique of LeGendre and Matsudaira [19]. The protein on the gel was transferred to the immobilon-psq PVDF membrane using 10 m*M* CAPS, 40% methanol buffer (pH 11.0) for 1 h at constant current (80 mA). The protein on the PVDF membrane was sequenced for 12 cycles according to Edman chemistry described below.

2.10. Edman sequencing

The sequencing technique was that of Edman [16], using an automated Beckman LF 3000 gas phase Protein Sequencer. Calibration was done with a Sigma Standard of 19 PTH-amino acids as suggested by the manufacturer. Samples of TSB-51 that were sequenced were obtained either from collection of CZE effluent (fraction collection) or immobilon-psq PVDF membranes as described above.

3. Results

Ohm's law plots of applied voltage vs. current were established to determine the optimum voltage for each buffer used in this study (data not shown). The optimum buffer-voltage combinations were 40 m*M* sodium phosphate buffer pH 2.5, 22.5 kV; 40 m*M* acetate buffer pH 4.0, 30.0 kV; 40 m*M* sodium phosphate buffer pH 7.0, 17.3 kV; 40 m*M* Tris–HCl buffer pH 8.0, 27.5 kV; and 40 m*M* sodium borate buffer pH 9.0, 25.0 kV. The applied voltage was chosen to keep the power at 3 W/m or less to allow for efficient cooling of the capillary during experiments.

3.1. Effect of voltage on the migration time of TSB-51

At constant temperature (25°C), increasing the applied voltage over the range 10 to 30 kV using 40 m*M* sodium borate buffer, pH 9.0, led to shorter migration times for TSB-51 and sharper peak resolution. For the experiment illustrated in Fig. 1,

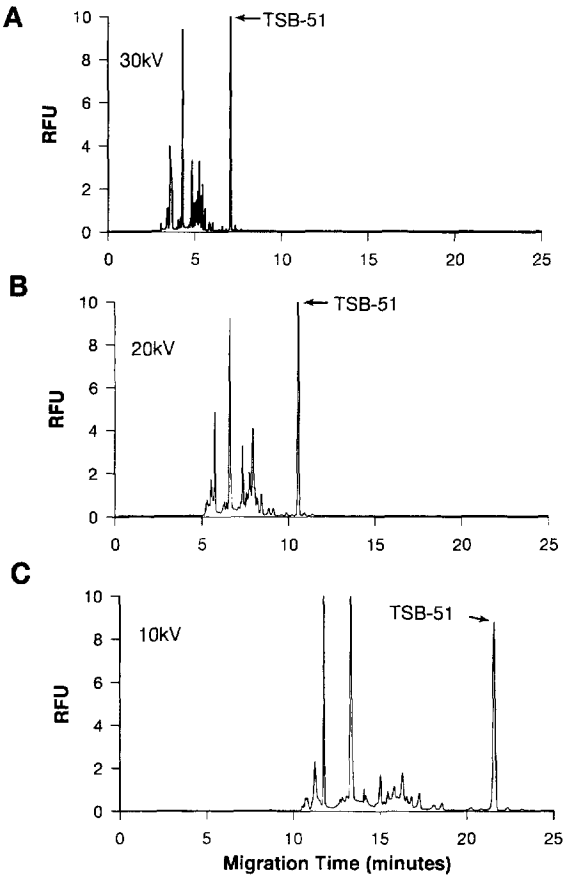

Fig. 1. Effect of voltage on migration time. After the *E. coli* lysate was derivatized with CBQCA, the sample (0.49 μ*M* TSB-51) was pressure injected for 4 s into an open uncoated silica capillary (57 cm×75 μm I.D.×375 μm O.D.). Different voltages were applied (10, 20 and 30 kV) using 40 m*M* sodium borate buffer pH 9.0 as the run buffer for 25 min at 25°C to separate TSB-51. Relative fluorescence units (RFU) were recorded at 520 nm.

applied voltages of 10, 20 and 30 kV generated migration times for TSB-51 of 21.58, 10.57 and 7.12 min, respectively.

3.2. Effect of pH on the migration time of TSB-51

The effect of pH on the migration time of the synaptobrevin–thioredoxin fusion protein is shown in Fig. 2. Increases in the pH of the run buffer led to decreases in the migration time of TSB-51 at constant temperature (25°C). In neutral to basic buffers of pH 7.0, 8.0 and 9.0, the observed migration times were found to be 19.54, 14.09 and 8.23 min, respectively. However, when acidic buffers (pH 2.5 or 4.0) were used, TSB-51 peaks were not detectable due to quenching of fluorescence of CBQCA.

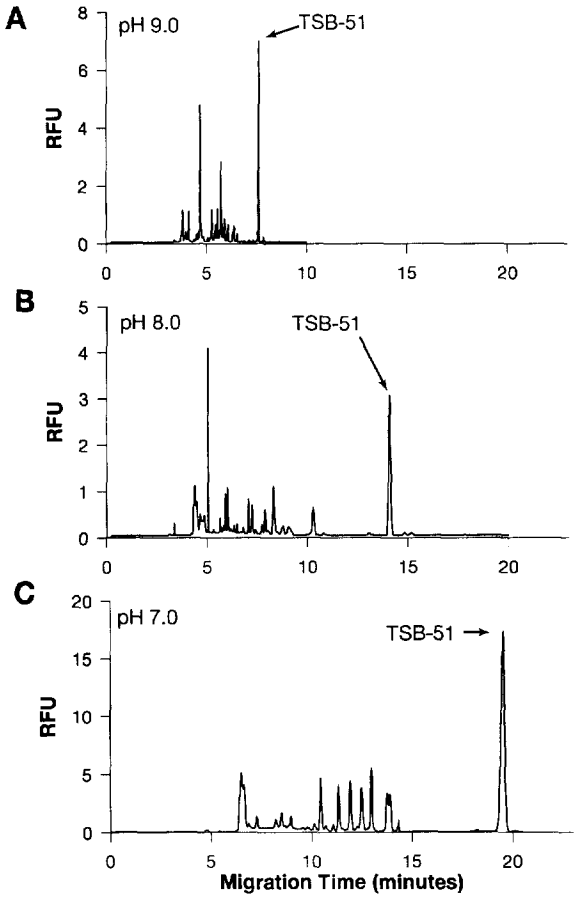

Fig. 2. Effect of pH on migration time. After the *E. coli* lysate was derivatized with CBQCA, the sample (0.49 μ*M* TSB-51) was pressure injected for 4 s into an open uncoated silica capillary (57 cm×75 μm I.D.×375 μm O.D.). Run buffers with different pH (40 m*M* sodium phosphate, pH 7.0; 40 m*M* Tris–HCl, pH 8.0; 40 m*M* sodium borate, pH 9.0) were used to separate TSB-51 with an applied voltage of 17.5, 27.5 and 25 kV. The separation was carried out at 25°C. Relative fluorescence units (RFU) were recorded at 520 nm.

3.3. Effect of temperature on the migration time of TSB-51

Temperature studies were performed to determine optimal capillary temperatures for separation of the synaptobrevin–thioredoxin fusion protein. The data in Fig. 3 indicate that as the temperature of the capillary increased, migration times decreased. At temperatures of 20 and 30°C, the migration times for TSB-51 were 7.60 and 5.88 min, respectively. The temperature-dependence of the migration time is thought to reflect alterations in the viscosity of the buffer. The optimal temperature to resolve TSB-51

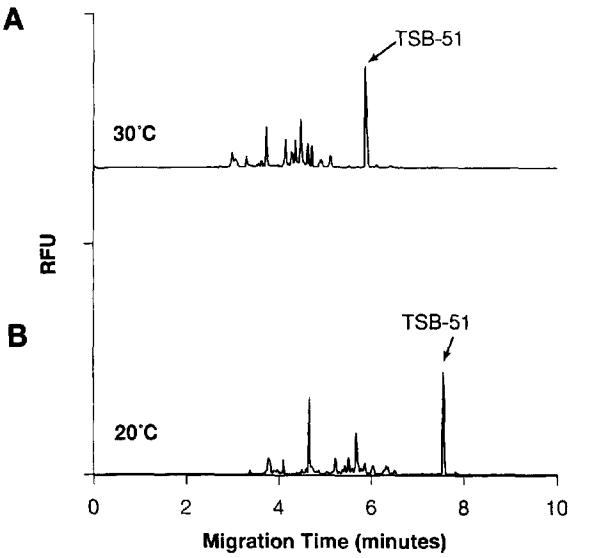

Fig. 3. Effect of temperature on migration time. After the *E. coli* lysate was derivatized with CBQCA, the sample (0.49 μ*M* TSB-51) was pressure injected for 4 s into an open uncoated silica capillary (57 cm×75 μm I.D.×375 μm O.D.). The run buffer was 40 m*M* sodium borate, pH 9.0 at 25 kV with temperatures of 20 or 30°C. Relative fluorescence units (RFU) were recorded at 520 nm.

was 25°C (Fig. 2A) since higher temperatures caused increased Joule heating.

3.4. Time course of CBQCA labelling of TSB-51

Studies to determine the time-course of CBQCA labelling of TSB-51 and of the marker dipeptide lysyl-glycine (30 n*M*) were performed to ensure that labelling conditions were optimal (Fig. 4). The labelling of lysyl-glycine and TSB-51 reached steady state at 1 and 4 h, respectively.

3.5. Fraction collection of TSB-51

The major peak identified as TSB-51 (Fig. 5, arrow) in the *E. coli* lysate was collected, for verification of its identity, during a capillary electrophoresis run using a constant voltage of 30 kV. As shown on the original electropherogram (Fig. 5), the migration time of TSB-51 was 6.77 min. The TSB-51 fraction that was collected after passage through the capillary and reinjected had a migration time of 6.76 min under the same experimental conditions (Fig. 5, inset). Note that the original solution exhibited 100 relative fluorescence units (RFU), while the fraction that was collected and reinjected had an RFU of only 0.25. The decrease in RFU is due to dilution of TSB-51.

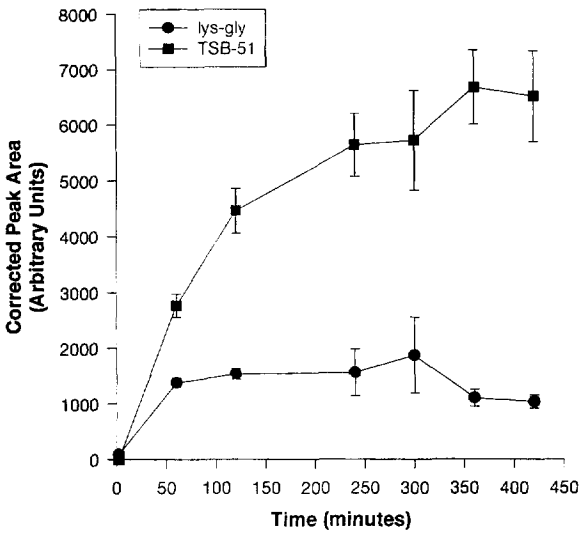

Fig. 4. CBQCA labelling of TSB-51. *E. coli* lysate containing TSB-51 (0.49 μ*M*) and lysyl-glycine (30 n*M*) were labelled in the same tube with CBQCA for 420 min at 25°C. At time 0, KCN was added to start the reaction. Sodium borate (40 m*M*) pH 9.0 was used as the run buffer to separate the fluorescent products. Every 60 min, a 4 s pressure injection of samples was carried out to monitor the labelling. After every injection, the capillary was rinsed for 2 min with 1 *M* NaOH and 1 min with 18 Ω water. Relative fluorescence units (RFU) were recorded at 520 nm.

3.6. SDS-PAGE gels and Edman sequencing

Amino acid sequencing by Edman degradation was performed for three cycles on the fraction collected from the TSB-51 peak, yielding SDK for the first 3 amino acids (data not shown). This corresponds to the first three amino acids of thioredoxin [5] and confirms the correct identity of the TSB-51 peak. Unfortunately, the small quantity collected precluded further sequencing of the fusion protein.

Because the yield of TSB-51 was so low, the fusion protein was also identified on a Tris–tricine SDS PAGE gel (Fig. 6). The band at ~19 kDa was transferred to a blot and also sequenced by Edman degradation. The result for 12 cycles of Edman degradation of the fusion protein was SDKIIHLTDDSF (data not shown), which coincides with the first 12 amino acids of thioredoxin [5]. The vector system (pTrxFus™) used for cloning synaptobrevin–thioredoxin fusion protein, links thioredoxin (S1–A108) to a linker (G109–G113), an enterokinase cleavage site (D114–K118), another linker (V119–P120) and the 51 amino acid fragment of synaptobrevin II (D121–K172) [6]. The expected sequence is SDKIIHLTDDSFDTDVLKADGAIL VDFWAEWCGPCKMIAPILDEIADEYQGKLT VAKLNIDQNPGTAPKYGIRGIPTLLLFKNGE VAATKVGALSKGQLKEFLDANLAGSGSGD DDDKVPDIMRVNVDKVLERDQKLSELDDR ADALQAGASQFETSAAKLKRKYWWKNLK.

The molecular mass of TSB-51 was found to be ~19 kDa (Fig. 6, arrow) on the SDS PAGE gel which is similar to the predicted molecular mass of 18.7 kDa using the program LASERGENE 1994 (DNASTAR, Madison, WI, USA). Note that the molecular mass of thioredoxin is 11.5 kDa [5], that of the linker region is 1.2 kDa and the synaptobrevin II fragment (51 amino acid) is 6.0 kDa, which comprises the total molecular mass observed on the gel. The data from

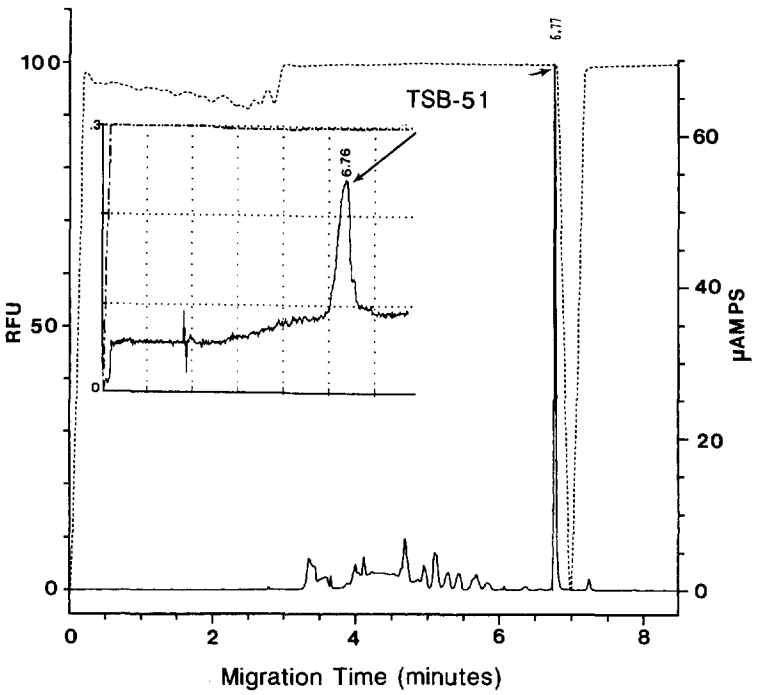

Fig. 5. Fraction collection of TSB-51 on CZE. After the *E. coli* lysate was derivatized with CBQCA, the sample (0.49 μ*M* TSB-51) was pressure injected for 4 s into an open uncoated silica capillary (57 cm×75 μm I.D.×375 μm O.D.). At 6.77 min, the voltage was automatically stopped (- - -), and the automated vial tray moved the collection microvial which contained 2 μl of 40 m*M* sodium borate buffer, pH 9.0, to the outlet of the capillary. After the collection vial was in place, the voltage was turned on (- - -) producing 69 μA. Electrophoresis continued allowing TSB-51 to be collected between 7–8.5 min at 25°C. Inset: The collected fraction of TSB-51 was pooled after 40 collections. This sample was reinjected to confirm that TSB-51 was actually collected. The migration time observed was 6.76 min (arrow), confirming the identity of the TSB-51 peak. The collected fraction of TSB-51 was sequenced by Edman degradation for 3 cycles yielding an N-terminal sequence of SDK. Relative fluorescence units (RFU) were recorded at 520 nm.

sequencing and SDS-PAGE gels confirm that the TSB-51 fusion protein was made successfully. Preliminary data from our laboratory show that this fusion protein is cleaved by BoNT/B light chain [6].

4. Discussion

This study is the first report of the TSB-51 fusion protein. Optimal conditions to identify and measure a synaptobrevin–thioredoxin fusion protein by CZE using LIF detection have been established. The protein was resolved from *E. coli* lysate using the CBQCA fluorophore, a run buffer consisting of 40 m*M* sodium borate at pH 9.0, a voltage of 25 kV and a capillary temperature of 25°C. Comparison of CBQCA and fluorescein–isothiocyanate (FITC) to label amino acids was made [20], and interfering peaks were obtained using FITC. Therefore, CBQCA was utilized in this study.

There are a number of advantages in measuring TSB-51 by the CZE–LIF method. First, CBQCA allows specific labelling of substances containing primary amines only, such as proteins, and does not label other substances that are present in the *E. coli* lysate. This is in contrast to HPLC measurement of all components present in an *E. coli* lysate that absorb in the wavelength range being used for detection. Second, the CZE–LIF detection method is approximately 1000-fold more sensitive than HPLC [21]. Third, by taking advantage of the short run times of CZE, kinetic studies of the endopeptidase activity of BoNT/B on TSB-51 can be easily performed. Samples may be injected automatically

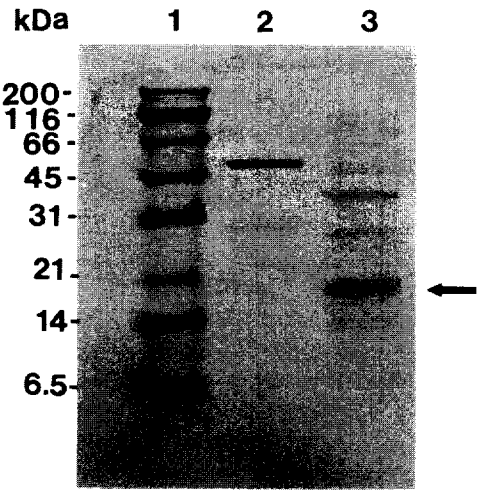

Fig. 6. Tris–tricine SDS-PAGE gel (10–20%) of TSB-51. Lane 1: broad range molecular mass markers: (kDa: 6.5, aprotinin; 14.4, lysozyme; 21.5, trypsin inhibitor; 31, carbonic anhydrase; 45, ovalbumin; 66.2, serum albumin; 97.4, phosphorylase B; 116, β-galactosidase; 200, myosin). Lane 2: pure BoNT/B light chain. Lane 3: The *E. coli* lysate (750 ng/lane). TSB-51 appears as an 18.7 kDa protein (arrow). This gel was blotted to an immobilon-psq PVDF membrane and sequenced. The first 12 amino acids for TSB-51 were confirmed.

during set intervals to follow the reaction progress. Finally, the use of CZE–LIF is cost-effective because the volume of reagents, such as custom-made peptides, utilized per injection (nanoliters) is 1000-fold less than that required for HPLC (microliters) [21]. Choosing a buffer (sodium borate, pH 9.0), an applied voltage to reduce Joule heating (25 kV) and optimal temperature (25°C) resulted in a fast, sensitive and reproducible separation assay.

The TSB-51 protein was derivatized using the fluorogenic reagent, CBQCA, over a 420 min period (Fig. 4). The protein was derivatized in the *E. coli* lysate buffer at pH 8.0. This agrees with the optimal pH range (8.0–9.5) in which CBQCA labels peptides of various lengths (4–9 aa) [15]. Liu et al. [15] were able to label small peptides (3–10 aa) with CBQCA within 1 h. The dipeptide lysyl-glycine was also labelled within 1 h, whereas complete labelling of TSB-51 required over 4 h (Fig. 4). The slower labelling of TSB-51 could be due to its longer length, compared to lysyl-glycine, or to its greater number of primary amine groups available for derivatization. Lysyl-glycine and TSB-51 may compete for labelling with CBQCA since they are derivatized in the same sample tube. The CBQCA complex of TSB-51 did not produce fluorescence in acidic buffers, thus confirming reports that the complex degrades at acidic pH [15]. The isoelectric point (p*I*) of the fusion protein was predicted to be 4.72 (LASERGENE 1994). In the basic buffer used (pH 9.0), TSB-51 has a net negative charge of −10.4 and migrates the fastest (Fig. 2A), compared to the other pH values studied. Identification of this peptide has been completed by fraction collection of the major peak on the electropherogram followed by Edman sequencing of the first 3 amino acids (Fig. 5). Due to a low yield of TSB-51 obtained by fraction collection, which did not allow sequencing beyond 3 cycles, the TSB-51 was separated by polyacrylamide gel electrophoresis, transferred to an immobilon-psq PVDF membrane and sequenced, showing that the first 12 amino acids of TSB-51 were SDKIIHLTDDSF. Therefore, the peak identified in Fig. 5 at 6.77 min is TSB-51. Use of CZE as a preparatory method for peptide and protein sequencing has been recently reported [22]. By coupling CZE–LIF with fraction collection and Edman sequencing, a recombinant fusion protein can be readily separated and identified as shown in this study.

Acknowledgments

The authors would like to acknowledge the technical support of Osman Haque and Chris Himmelheber. We would also like to thank Tracey Hamilton for production of gel photographs for this manuscript.

References

[1] L.L. Simpson, Annu. Rev. Pharmacol. Toxicol. 26 (1986) 427.
[2] G. Schiavo, F. Benfenati, B. Poulain, O. Rossetto, P. Polverino de Laureto, B.R. DasGupta, C. Montecucco, Nature 359 (1992) 832.
[3] M. Baumert, P.R. Maycox, F. Navone, P. De Camilli, R. Jahn, EMBO J. 8(2) (1989) 379.
[4] T.C. Sudhof, Nature 375 (1995) 645.
[5] A. Holmgren, Annu. Rev. Biochem. 54 (1985) 237.

[6] L.J. Nowakowski, B.C. Courtney, Q.A. Bing, M. Adler, Anal. Biochem. (in press).
[7] P. Fora, C.B. Shone, J.O. Dolly, Biochemistry 33 (1994) 15365.
[8] C.B. Shone, C.B. Quina, R. Wait, B. Hallis, S.G. Fooks, P. Hambleton, Eur. J. Biochem. 217 (1993) 965.
[9] P. Washbourne, G. Schiavo, C. Montecucco, Biochem. J. 305 (1995) 721.
[10] W.S. Trimble, J. Physiol. 87 (1993) 107.
[11] L. Edelmann, P.I. Hanson, E.R. Chapman, R. Jahn, EMBO J. 14 (1995) 224.
[12] N. Calakos, M.K. Bennett, K.E. Peterson, R.H. Scheller, Science 263 (1994) 1146.
[13] F. Cornille, N. Goudreau, D. Ficheux, H. Niemann, B.P. Roques, Eur. J. Biochem. 222 (1994) 173.
[14] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298.
[15] J. Liu, Y.Z. Hsieh, D. Wiesler, M. Novotny, Anal. Chem. 63 (1991) 408.
[16] P. Edman, Acta Chem. Scand. 10 (1956) 761.
[17] H.J. Boss, M.F. Rohde, R.S. Rush, Anal. Biochem. 230 (1995) 123.
[18] H. Schagger, G. von Jagow, Anal. Biochem. 166 (1987) 368.
[19] N. LeGendre, P. Matsudaira, Biotechniques 6 (1988) 154.
[20] B.J. Wanders, A.A. van de Goor, Application #DS826, Beckman Instruments, 1992.
[21] B.L. Karger, A.S. Cohen, A. Guttman, J. Chromatogr. 492 (1989) 585.
[22] N.J. Kim, J.H. Kim, K.-J. Lee, Electrophoresis 16 (1995) 510.