Food Control 46 (2014) 282–290

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Enhanced immunodetection of bovine central nervous tissue using an improved extraction method

Qinchun Rao [a], Yun-Hwa Peggy Hsieh [b,*]

[a] Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108, USA
[b] Department of Nutrition, Food and Exercise Sciences, Florida State University, Tallahassee, FL 32306-1493, USA

ARTICLE INFO

Article history:
Received 21 December 2013
Received in revised form
29 April 2014
Accepted 26 May 2014
Available online 6 June 2014

Keywords:
Bovine spongiform encephalopathy
Central nervous tissue
Myelin basic protein
Immunoassay
ELISA
Extraction

ABSTRACT

The infectivity of bovine spongiform encephalopathy (BSE) was mainly associated with bovine central nervous tissue (CNT), which have been banned from food and feed chain in many countries. Detection of bovine CNT is important for the surveillance of BSE occurrence. Myelin basic protein (MBP) has been identified as a thermal-stable maker protein in CNT for the detection of CNT in processed meat and feedstuffs. This study reports an improved extraction method of MBP that substantially enhanced the detectability of bovine CNT.

Two different buffers (10 mM phosphate buffered saline and 20 mM Tris) at neutral pH containing different salt concentrations (150 mM–1 M) were used to extract proteins from unheated and heated (100 °C for 30 min) bovine brain. The mixtures were extracted with different time periods (30–180 min) at room temperature. Results from indirect non-competitive enzyme-linked immunosorbent assay (ELISA), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot show that several factors (buffer type, salt valence and concentration, incubation time, and heat treatment) affected the MBP concentration in the extracts. The concentration of MBP increased with the increase of salt concentration in all extracts, but decreased with the increase of the incubation time in unheated samples. MBP was more effectively extracted with the buffers containing divalent salt (Ca^{2+} or Mg^{2+}) than that of monovalent salt (Na^{+}). The MBP concentration was higher in heated than unheated samples. The optimal method for MBP extraction was using 20 mM Tris–HCl containing 600 mM $MgCl_2$ (pH 7.4) to extract heated (100 °C for 30 min) CNT samples for 1 h at room temperature. With this sample extraction method, the detection limit of an indirect competitive ELISA can be significantly improved from reported 10%–0.05% (g/g) of bovine brain tissue.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Since the European Union (EU) banned the use of proteins derived from mammalian tissues for feeding ruminants in 1994 (European Commission, 1994), the number of bovine spongiform encephalopathy (BSE) cases reported around the world has been decreased significantly year by year. The major reason is because the enforcement of this regulation in Europe, especially in the United Kingdom which has reported about 97% BSE cases worldwide (World Organisation for Animal Health, 2013), essentially interrupts the spread of infection of the BSE agent, abnormal prion (PrP^{Sc}), in the cattle through the animal feed chain. To further eliminate the risk of cross-contamination of cattle feed with feed intended for other animals and which contains animal proteins possibly contaminated by the BSE agent, the EU has introduced a total ban since 2001 on the feeding of processed animal protein to farm animals which are kept, fattened or bred for the production of food (European Commission, 2000). Although currently the BSE has been under control, the worldwide surveillance is critically important to prevention of the reoccurrence and the feed regulation continues. Two specified risk materials (SRM) in bovine central nervous tissue (CNT), brain and spinal cord, which harbor the highest concentration of PrP^{Sc} are associated with about 90% of the BSE infectivity (European Commission's Scientific Steering Committee, 1999). In North America and the EU, currently, the central nervous system (CNS) based SRM has been prohibited for both human food and animal feed (Canada Gazette, 2003; European Commission, 1997; U.S. Food and Drug Administration, 2008). In order to enforce these regulations and finally extinguish BSE, it is necessary to develop a reliable method for the detection of CNT contamination in meat and feed products.

* Corresponding author. Tel.: +1 (850) 644 1744; fax: +1 (850) 645 5000.
E-mail address: yhsieh@fsu.edu (Y.-H.P. Hsieh).

http://dx.doi.org/10.1016/j.foodcont.2014.05.040
0956-7135/© 2014 Elsevier Ltd. All rights reserved.

Although many detection methods for CNT in meat have been developed, none of these methods are useful for analyzing processed meat products, let alone severely heat-treated feed products (133 °C for 20 min), such as meat and bone meal (MBM). In order to develop a reliable and effective detection method, identifying a heat-stable marker from CNT is critical for the assay development. MBP has been used as a marker for the detection of CNT contamination using immunoassay in a number of studies (Herde, Bergmann, Lang, Leiser, & Wenisch, 2005; Rencova, 2005; Sultan et al., 2004; Tersteeg, Koolmees, & van Knapen, 2002). Our preliminary data showed that at neutral pH, bovine MBP can maintain its molecular integrity after heating at 100 °C for 30 min, while its immunoreactivity stays clearly detectable even after severe heat treatment (133 °C for 30 min) (unpublished data).

However, based on the detection of MBP, except for immunohistochemistry (Tersteeg et al., 2002), two reported immunoassay techniques, ELISA (Rencova, 2005) and immunoblotting (Herde et al., 2005; Sultan et al., 2004), showed high detection limit of CNT (from 1 to 10%, g/g). Both assays used buffers to extract the target analyte (MBP) before the analysis, one of the major reasons for the poor detection limit of the assay could be the low concentration of MBP in the sample extracts. In both studies (Rencova, 2005; Sultan et al., 2004), sodium dodecyl sulfate (SDS), an anionic detergent, was one of the major ingredients in their MBP extraction buffers. It has been reported that the interaction between MBP and SDS involves the initial ionic interaction of the detergent with protein resulting in aggregation and turbidity in the solution. Within the aggregated complexes, molecules may rearrange to maximize hydrophobic interactions (Jones & Rumsby, 1978). The poor MBP yield of these extraction buffers could subsequently lead to the poor CNT detection limit of the assays. Therefore, in this study, we attempted to develop an improved extraction method to increase the concentration of MBP in the extracts, thus may further enhance the detectability of CNT in processed samples. The effects of extraction buffer types, the different buffer salt valences and concentrations, heat treatment and extraction time will be investigated. Once the MBP extraction method has been developed, we will evaluate its efficiency of MBP extraction by comparing the detectability of MBP in an indirect competitive ELISA (icELISA) using the identical commercial anti-MPB-monoclonal antibody (mAb) as Rencova (2005) reported for a comparison.

2. Materials and methods

2.1. Materials

Bovine and porcine CNT (brain and spinal cord) were obtained from the National Animal Disease Center (Ames, IA) and the Meat Processing Center at the University of Florida (Gainesville, FL), respectively. Horse brain and spinal cord were obtained from ELISA Technologies, Inc. (Gainesville, FL). Deer and elk brains were purchased from Grande Premium Meats (Del Norte, CO). Bovine peripheral nervous system (PNS) tissue (dorsal root ganglion) and brains from different species (canine, chicken, rabbit and rat) were purchased from Pel-Freez, LLC. (Rogers, AR). Goat brain was obtained from a local farm in Quincy, FL. Soybean meal was obtained from Say Best/Grain States Soya, Inc. (West Point, NE). Beef and pork loin were purchased from local supermarkets. All tissues were stored at −80 °C until use.

Bovine serum albumin (BSA), calcium chloride ($CaCl_2$), glycine, hydrogen chloride (HCl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), sodium chloride (NaCl), sodium dodecyl sulfate (SDS), trichloroacetic acid (TCA), tris(hydroxymethyl)aminomethane (Tris) and Tween 20 were purchased from Fisher Scientific (Fair Lawn, NJ). Alkaline phosphatase (AP) conjugate substrate kit, goat anti-rat IgG (H + L) AP (anti-IgG-AP) conjugate, 20 mM Tris-buffered saline (TBS, pH 7.5), and Precision Plus Protein Kaleidoscope standards were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA). 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), goat anti-rat IgG (Fc specific) horseradish peroxidase (anti-IgG-HRP) conjugate, monoclonal anti-myelin basic protein (MBP) antibody produced in rat (anti-MBP mAb), and EZBlue gel staining reagent were purchased from Sigma–Aldrich Co. (St. Louis, MO). Methanol was purchased from VWR International (West Chester, PA). All chemicals and reagents were analytical grade. All solutions were prepared using distilled-deionized, and sterilized pure water from the NANOpure Diamond ultrapure water system (Barnstead International, Dubuque, IA).

2.2. Selection of CNT protein extraction method

To effectively increase the concentration of MBP in the sample extracts, different CNT protein extraction buffers were studied. The detailed composition of these buffers is listed in Table 1. Quantitative amounts of raw bovine brain (approximately 15 g) with a 1:1 ratio (g/g) were homogenized with pure water at 4 °C (2 min at 11,000 rpm and 1 min at 13,000 rpm) using the ULTRA-TURRAX T25 basic homogenizer (IKA Works, Inc., Wilmington, NC). The homogenate was equally divided into 1.5-ml microcentrifuge tubes (200 μl each) and kept at −80 °C until use.

After thawing at room temperature for 10 min, the aliquots of homogenate were heated at 100 °C for 30 min. The heated samples were cooled by immersing in ice-cold water immediately and then were added to 400 μl diverse extraction buffers, respectively. Another set of unheated aliquots were thawed and added to 800 μl of these buffer solutions, respectively. All samples were mixed at room temperature on the analog vortex mixer (VWR) at speed 9 for 1 min and then rotated end-over-end at room temperature with different extraction times (30–180 min) on the rocker (Labnet International, Inc., Woodbridge, NJ) at 45 rpm. After extraction, the mixtures were centrifuged at 16,000 × g for 5 min at 4 °C. The pellets and supernatants were separated and the supernatants were centrifuged again at the same speed and temperature for 5 min before the analysis. The protein concentration of the samples was determined using the Protein Assay Kit II (Bio-Rad) according to the method of Bradford (1976). Bovine 18.5-kDa MBP was purified according to the published procedure (Beniac et al., 1997) and used as protein standard.

2.3. SDS-PAGE and Western blot

To examine the MBP extraction ability of different extraction buffers, unheated and heated bovine brain samples extracted with different buffers were separated by SDS-PAGE (5% stacking gel and 12% separating gel) using the Mini-Protean III Electrophoresis Cell (Bio-Rad) according to the method of Laemmli (1970). Before running SDS-PAGE, the protein samples to be analyzed were gone through the TCA precipitation to remove interfering salts (Rosenberg, 2005). Briefly, the volume of the protein extract was adjusted to a final concentration of 20% (ml/ml) TCA using the 100% (g/ml) TCA stock solution (add 227 ml of H_2O to a bottle containing 500 g TCA). The mixture was then incubated on ice for 30 min. After the centrifugation (16,000 × g for 5 min at 4 °C), the supernatant was discarded. To remove the TCA, the pellet was rinsed with 200 μl cold acetone then centrifuged at 16,000 × g for 5 min. The protein precipitate was dissolved in 5% SDS sample buffer and heated for 5 min in boiling water (100 °C). The samples were analyzed by SDS-PAGE (5% stacking gel and 12% separating gel). The running buffer

Download English Version:

https://daneshyari.com/en/article/6391226

Download Persian Version:

https://daneshyari.com/article/6391226

Daneshyari.com