Isolation and Characterization of Full-Length Recombinant Cattle PrP^C Protein

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 1, pp. 68-71, January, 2006
Original article submitted June 20, 2005

Full-length *Bos taurus* PrP^C protein was obtained in the eu- and prokaryotic expression systems. Immunoblotting and indirect enzyme immunoassay demonstrated high specificity and antigenic activity of full-length proteins in the reactions with monoclonal antibodies (anti-SAF-32 and VRQ-84). Membrane location of recombinant PrP^C protein in insect cells was shown by immunofluorescent analysis.

Key Words: *prion; recombinant protein; expression*

Transmitted spongiform encephalopathies (TSE) are a group of human and animal lethal neurodegenerative diseases, including Creutzfeldt—Jakob disease, Gerstmann—Straussler—Scheinker syndrome, kuru, cattle spongiform encephalopathy (CSE), sheep scrapie, mink transmitted encephalopathy, and some other diseases [1].

The concept of protein nature of TSE agent is universally acknowledged [7]. The disease is believed to be associated with conformation transition of normal cellular PrP^C protein into abnormal PrP^d form (PrP^{res}, PrP^{Sc}) with increased content of sites with β-sheet conformation [5,6]. The disease develops because of accumulation of this proteinaze-resistant abnormal protein isoform, called prion.

The development of rapid and effective methods for detection of prion protein in biological fluids, essential for early life-time diagnosis, is now in progress. Immunological methods for detection of PrP^d are developed most intensely [2]. The possibility of *in vivo* blocking PrP^C conversion into PrP^d for preventing disease development is investigated [11]. Preparation of recombinant PrP^C protein opens new vistas for the development of immunological methods for its detection in biological material and for *in vitro* studies of conformation transition of PrP^C molecule into PrP^d. This latter can be used in the search for agents blocking this transition *in vivo*.

The aim of our study was to obtain a recombinant PrP^C, evaluate its immunological characteristics and prospects for diagnostic use, and determine its subcellular location.

MATERIALS AND METHODS

Genome DNA isolated from *Bos taurus* blood served as the source of gene amplification. Constructs for expression of recombinant PrP^C protein in eukaryotic (baculovirus) "Bac-to-Bac" (Invitrogen) and prokaryotic pET-system (Novagen) systems were obtained by PCR and molecular cloning. The expression in eu- and prokaryotic systems was carried out as recommended by the manufacturers. Insect Sf-21 cells were used for transfection, Sf-21 and High-Five cells for accumulation of preparative amounts of baculovirus PrP^C. A panel of 19 anti-PrP^C monoclonal antibodies (MAB) was used: 1-Surg, 2pr-Surg, 2m-Surg, 3-Surg, 4G-11, 4G-29, 4G-57, 4G-69, 5G-13, 5G-29, 5G-38, 5G-48, 5G-

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0007-4888/06/1411-0062

55, 5G-77, obtained to synthetic Prionics Ag peptide (NARVAC Company); anti-SAF-32 and anti-PrP^C-Hum (gracious gift from J.-F. Deslie, CEA); and VRQ-14(33), VRQ-84, and PLT-40(2), kind gift from J. Grossklaude, INRA. Recombinant proteins were purified using Ni-NTA-agarose (Qiagen) in accordance with the instruction. A 3-day monolayer of Sf-21 cells infected with a recombinant baculovirus carrying PrP^C was stained with an immunofluorescent dye.

RESULTS

We assumed that full-length $recPrP^C$ obtained in baculovirus system of expression was glycosylated, contained maximum number of antigen epitopes, and was highly immunocompetent, because post-translation modifications in insect cells were similar to those in mammals [4]. That is why it was selected for testing with a panel of MABs. The panel consisted of 19 MABs to fragments and full-length cattle, sheep, and human PrP^C and to brain suspension of a hamster infected with adapted scrapie strain. The resultant crude full-length protein was characterized by high specificity and antigenic activity in the reactions with anti-SAF-32 and VRQ-84 MABs (Fig. 1).

The location of full-length $recPrP^C$ in insect cells was detected by indirect immunofluorescent analysis using VRQ-84 antibodies. Full-length $recPrP^C$ was present mainly in the cell membrane, which was seen from bright contour of specific fluorescence (Fig. 2). This location of PrP^C is characteristic of mammalian cells [3]. Therefore, the synthesis, processing, and intracellular transport of recombinant protein in insect cells are similar to those in mammalian cells. Hence, this cell system for expression of PrP^C protein can be used for investigation of the subcellular location, immunological and physicochemical characteristics of the protein.

At the next stage of the study crude $recPrP^C$ proteins obtained in eu- and prokaryotic expression systems of were characterized in immunoblotting with anti-SAF-32 antibodies (Fig. 3). Immunoblotting of PrP^C of baculovirus origin showed, in addition to the main band (mol. weight 27-29 kDa), additional high molecular-weight bands, various glycoforms [8], and low molecular-weight products, emerging as a result of partial proteolytic degradation [10]. A lower molecular weight of the baculovirus product in comparison with mol. weight of $recPrP^C$, expressed in the prokaryotic system (30 kDa), seems to be due to removal of PrP^C signal sequence in insect cells, in contrast to *E. coli* cells where eukaryotic protein is not processed. Testing of recombinant products in indirect EIA with anti-SAF-32 and VRQ-84 gave similar results: antigens exhibited high activity and specificity of antibody binding, this indicating the absence of glycosylation effect on immunocompetence of $recPrP^C$ protein.

Immunospecificity of fractions obtained by metal-affinity chromatography with Ni-NTA-agarose products was evaluated using anti-SAF-32 or VRQ-84 MAB by EIA and immunoblotting. The yield of the product under native conditions was lower than under denaturing (8 M urea) conditions. Further purification was carried out only under denaturing

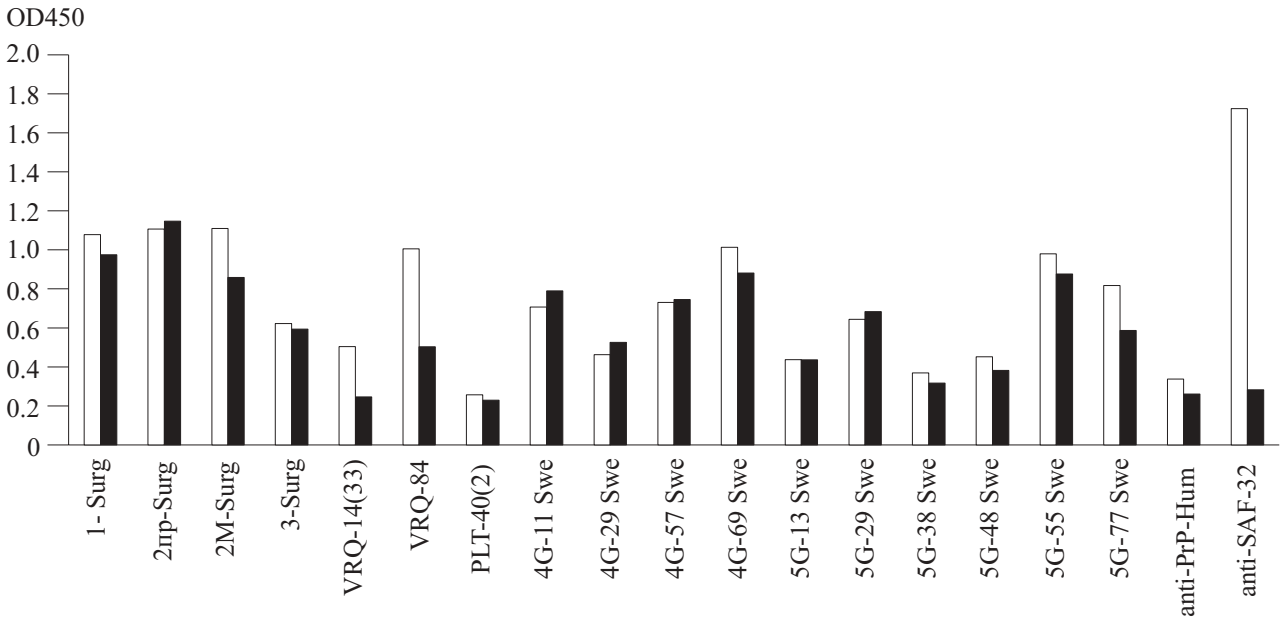

Fig. 1. Testing of a panel of monoclonal antibodies in indirect enzyme immunoassay with full-length cattle prion protein obtained in the baculovirus system of expression. Light bars: Sf-21 cells infected with recombinant baculovirus; dark bars: non-infected Sf-21 cells.

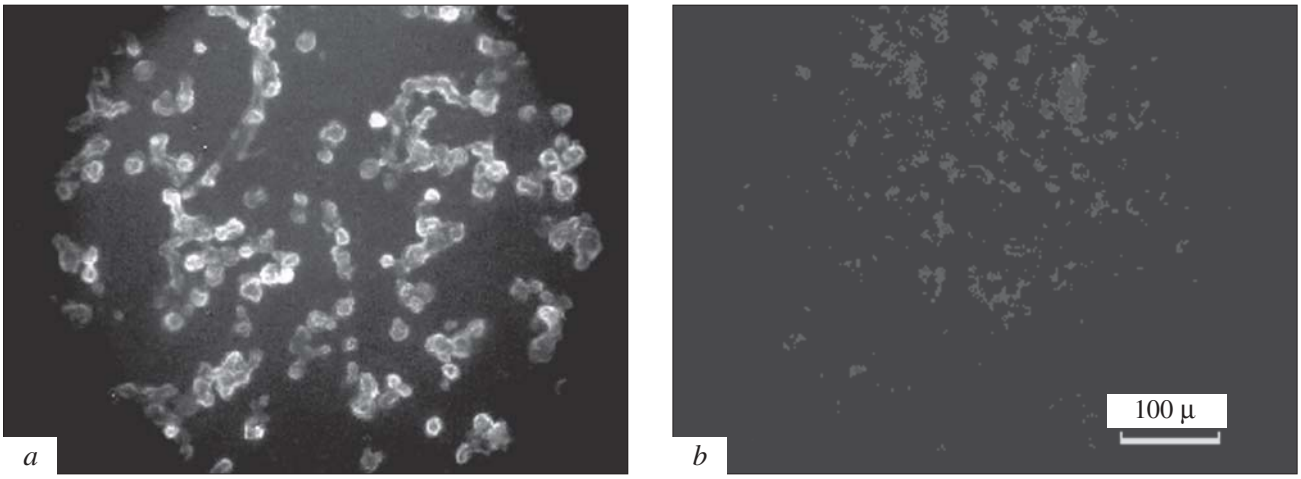

Fig. 2. Immunofluorescent staining of Sf-21 cells expressing the coding sequence of full-length cattle PrP^C protein. *a*) Sf-21 cells infected with recombinant baculovirus; *b*) negative control (Sf-21 cells infected with wild type baculovirus).

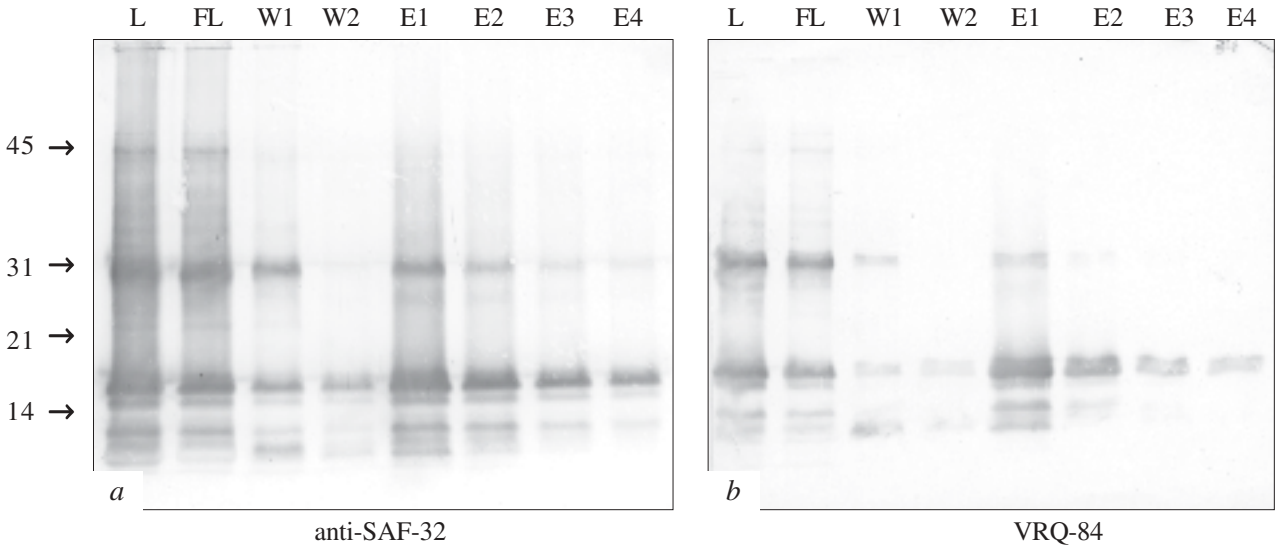

Fig. 3. Detection of recPrP of baculovirus origin by immunoblotting with anti-SAF-32 (*a*) and VRQ-84 monoclonal antibodies (*b*) in protein fractions purified by metal affinity chromatography on Ni-NTA-agarose. L: cell lysate; FL: free protein; W1-W2: washing buffer (pH 6.3); E1-E4: elution buffer (pH 4.5). Arrows show reference mol. weights.

conditions by the Qiagen protocol. The bulk of immunocompetent PrP was eluted from the column at pH 5.9-4.5, the eluates contained, along with full-length PrP with a mol. weight of 27-29 kDa, appreciable amounts of immunocompetent product with a mol. weight of about 17-19 kDa. The appearance of a low-molecular-weight protein seems to be due to partial degradation of full-length protein. Further use of proteinase inhibitor mixtures (Sigma) prevented proteolytic degradation of full-length baculovirus product and increased its output. Distribution of purified prokaryotic PrP^C protein by fractions was similar. The resultant fractions contained no proteolytic degradation products (even without proteinase inhibitor treatment). It seems that amino acid sequence of PrP^C protein carries no recognition sites for *E. coli.* proteinases. The expression of recombinant protein in the prokaryotic system was significantly higher than in baculovirus system.

Presumably, glycosylation has no appreciable effect on immunological recognition of recombinant PrP^C by specific MAB. The process of PrP^C to PrP^d conversion depends on the presence of sugars [9], and therefore conditions of *in vitro* conformation transition can be different for the resultant polypeptides. Higher level of expression in *E. coli* and absence of differences in antigenic activities of recombinant products are advantages suggesting the use of prokaryotic antigen in experiments re-

quiring appreciable amounts of recPrP^C. Our present findings and published data [1,7,8] indicate the possibility of using variants of *B. taurus* recombinant PrP^C as antigens for obtaining specific anti-PrP^C/PrP^d monoclonal antibodies, which can be useful in the development of approaches to the diagnosis of CSE. In addition, creation of a model for *in vitro* studies of PrP^C/PrP^d conformation transitions and understanding of the mechanism of this process are essential for further studies of therapeutic agents blocking the formation of infectious isoform of the prion in the body [11].

The study was carried out within the framework of MNTC 2777p Project in collaboration with Professor Donald Knowlus and Dr. Catherine O'Rourke (Department of Agriculture of the USA, University of Washington), to whom the authors are deeply grateful for consultations and support.

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