ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Research of Methods of Identification and Quantitative Content of Prion Protein in Blood of Animals and Man.

Andrei Lisitsyn[1]*, Alexander Prosekov[2], and Olga Kriger[2].

[1]Federal State Scientific Institution "All-Russian Research Institute of Meat Industry. VM Gorbatov" 109316, Moscow, Russia. Talalikhina 26.
[2]Federal State-owned Budgetary Educational Institution of Higher Vocational Education "Kemerovo Institute of Food Science and Technology", 47 Stroiteley Boulevard, 650056 Kemerovo.

ABSTRACT

The following prion sequences have been chosen for the present work: a domestic horse, a donkey, a pig, a cow and other animals and also a man. For the protein identification, mass spectrum of its peptide map was received after hydrolysis by trypsin. The carried out research shows that the investigated samples are indeed normal pathogenic proteins of cattle. The quantitative content of normal prion protein in the test samples was estimated with the help of protein electrophoresis using the Laemmli method with the subsequent coloration of Coomasie Brilliant Blue R250 gel. The executed phylogenic analysis confirmed that the sequences of prion protein gene are highly conservative and differ only with conformation and proteolysis resistance connected with it. That is why it is impossible to choose a DNA-target from the prion sequences for the subsequent analysis with polymerase chain reaction.

Keywords: protein, blood, domestic animals

Corresponding author

ISSN: 0975-8585

INTRODUCTION

According to the statistical data, Creutzfeldt-Jacob disease annually affects about 150 people in Russia with its population of about 150 million people. However, the account of such patients is held very badly: the disease cases are neither diagnosed nor registered [1].

A constantly increasing theoretical interest to the problem is brought about by the results of biomolecular analysis of prion protein structure, that allowed to gather and to a great extent systemize significant information concerning the structure, functions and accumulation of these infectious agents in the infected organism of a man and animals [2].

It is the results of biomolecular analysis of prion protein structure that afforded ground for marking new directions in further approaches to the diagnostics and therapy of prion diseases [3].

A significant number of methods are used to analyse protein substances, including different modifications of Kjeldahl method, photometric methods using spectrophotometers and photoelectric colorimeters [4].

The biuret reaction has gained a wide acceptance among accelerated photometric methods [5].

METHODS

The following prion PRNP sequences have been chosen for comparing: *Equus caballus* (a domestic horse), *Equus asinus* (a domestic donkey), *Sus scrofa* (a pig), *Bos taurus* (a cow), *Bos javanicus* (a Javanese bull), *Bubalus bubalis* (a buffalo), *Syncerus caffer caffer* (an African buffalo), *Capra hircus* (a goat), *Ammotragus lervia* (a Barbary wild sheep), *Ovis aries* (an urial), *Rangifer tarandus granti* (a reindeer), *Capreolus capreolus* (a roe deer), *Alces alces alces* (a moose), *Cervus elaphus nelsoni* (a North-American moose), *Cervus dama* (a fallow deer), *Homo sapiens* (a man).

The following works have been carried out to determine the prion protein normal form: one-dimensional electrophoresis execution, protein cleavage with trypsin in gel, protein identification by the method of peptide masses fingerprint.

Two protein samples were used as investigation objects, determined during the investigation as highly infective in respect of developing a pathogenic form of the prion protein. The samples that have been chosen for carrying out the investigation are given in Table 1

Table 1 – Protein samples for investigation

No.	Source	Protein Mass, kDa
1	Blood serum	34,89
2	Meat proteins water-soluble fraction	32,38

For the protein identification, mass spectrum of its peptide map is received after hydrolysis by trypsin [6]. The spectrum is received within the mass range of 700-4500 m/z, choosing the laser capacity optimal for having the best resolution, trying to get trypsin autolysis peaks in the spectrum for an opportunity of further internal calibration. The mass spectra are developed with the software package FlexAnalysis 2.4 (Bruker Daltonics, Germany). Where necessary, spectra of separate peptides fragmentation were received in tandem mode. Possible marking of amino acid sequences is made for successful mass spectra of fragmentation [7].

Phylogenic connections between living organisms can be laid by way of comparing genes sequences and separate genes sections coding ribosomal ribonucleic acids (RNA). The data of completely or partially sequeneed genes of RNA of different microorganisms get into international databases and are available via the computer network. At present, the methods based upon determination of the nucleotide sequence of the ribosomal genes are widely used for the specific identification of different infections [8].

Polymerase chain reaction (PCR) is an experimental method that allows us to gain a significant

ISSN: 0975-8585

increase of weak concentrations of certain fragments of desoxyribonucleic acid (DNA) in the investigated test sample. The base of the PCR method is a natural process, which is a complementary completion of the DNA matrix performed with the DNA-polymerase ferment.

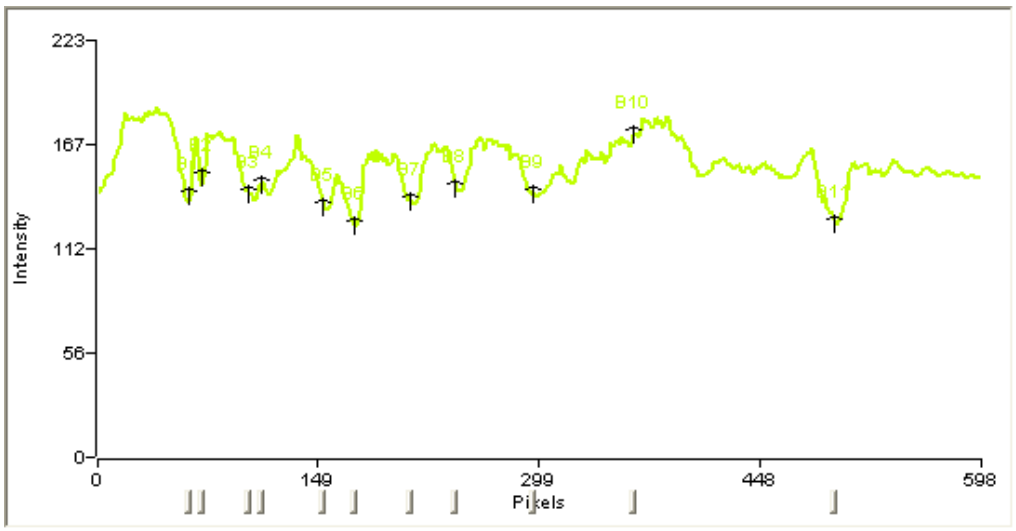
To choose a DNA-system, a kind of PCR has been chosen, which is a real time immuno-PCR, to detect infectious prion proteins, where the DNA molecule is used as a marker. Immuno-PCR allows detection of the pathogenic prion protein using specific antibodies marked by a double-stranded DNA.

Immuno-PCR combines the enzyme-linked immunoassay versatility with the capacity and sensitivity of PCR.

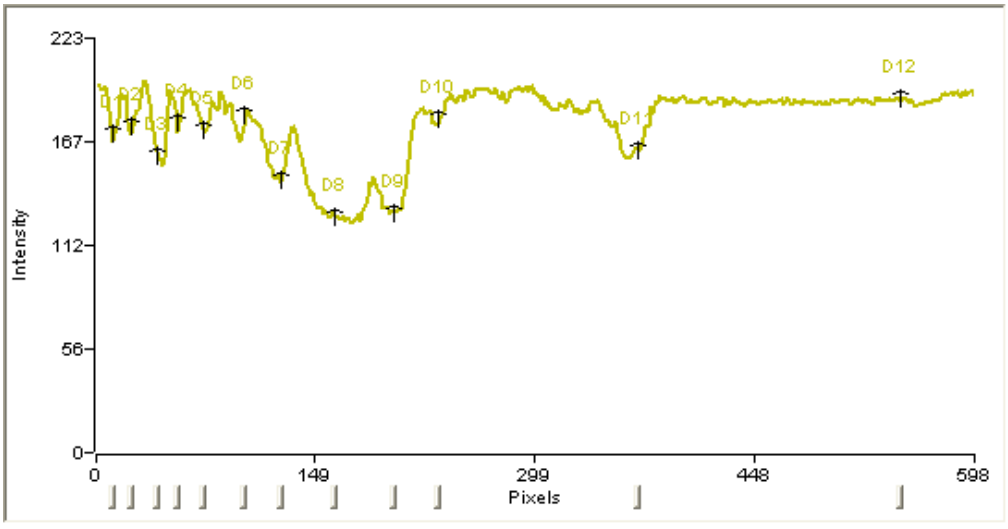
RESULTS

The carried out research shows that the investigated samples are indeed normal pathogenic proteins of cattle. The quantitative content of normal prion protein in the test samples was estimated with the help of protein electrophoresis by Laemmli method with subsequent coloration of Coomasie Brilliant Blue R250 gel.

The received electrophoregrammes of meat water-soluble fraction and blood plasma are given in Picture 1.



a)



b)

Figure 1. Biogenic test samples electrophoregrammes:
a) meat water-soluble fraction; b) blood plasma.

ISSN: 0975-8585

The calculation of the protein concentration in the test sample was made according to the formula:

$$C=(Cp*Cf)/100,$$

where Cp – content of the total protein in the test sample, g/100g;

Cf – content of the protein fraction from the total protein content in the test sample, g/100g of protein.

The experimental data, received during the experiment, are given in Table 2.

Table 2 – Protein fraction composition in the investigated test samples

Strip No.	Molecular weight, kDa	% from total protein content	% from total protein content in the test sample	Protein concentration, g/100 g of the test sample
B 7	37.42	18.19	10.78	0.74
B 8	32.38		7.41	0.51
D 9	39.17	22.06	21.03	2.05
D 10	34.89		1.03	0.1

Relative content of protein fractions with the weight from 30 to 40 kDa in the water-soluble fraction of meat proteins makes 18.19% from the total quantity, it is 1.25 g/100 g of meat.

The received electrophoregrammes of the blood plasma test samples show that relative content of proteins with the weight from 30 to 40 kDa in the blood plasma makes 22.06% from the total quantity, which is 2.15 g/100 g of blood plasma. A significant number of methods is used to analyse protein substances, including different modifications of Kjeldahl method, photometric methods using spectrophotometers and photoelectric colorimeters.

The biuret reaction has gained a wide acceptance among accelerated photometric methods.

To carry out investigations on extraction of animal proteins, samples of tissues from cattle and animal origin products were prepared, animal proteins fractionating was executed, optical density was measured, calculations were made. Final results of protein contents in different fractions are given in Table 3.

Table 3 – Protein content in different fractions

Sample	Protein content						Total	
	Water-soluble		Salt-soluble		Alkali-soluble			
	mg/g	%	mg/g	%	mg/g	%	Mg/g	%
Low-fat beef	648,32	6,48	811,41	8,11	324,28	3,24	1784,01	17,83
Brain (grey substance)	-	29,34	-	-	-	4,95	-	-
Brain (white substance)	-	19,13	-	-	-	21,97	-	-

According to Table 3 data, the most protein quantity is contained in the water-soluble and salt-soluble fractions of low-fat beef.

The total protein content in the test samples was determined by the procedure of measuring mass fraction of total and protein nitrogen in meat, meat products and protein containing food items by Dumas method of burning.

ISSN: 0975-8585

Table 4 – The total quantitative content of protein in the samples

Investigation object	Weight, mg	Total nitrogen content, %	Conversion factor	Total protein, %	Measurement error, ±δ,%
Full milk	195,60 192,40	0,576 0,558	4,64	2,63	0,31
Blood serum	188,12 180,72	2,370 2,374	6,25	14,83	0,89
Whole blood	214,50 254,80	3,462 3,571	6,25	21,97	1,32
Cheese	189,70 229,60	3,328 3,506	4,64	15,85	0,95
Low-fat beef	126,50 110,00	3,222 3,347	5,62	18,46	1,11
Water-soluble fractions	97,40 96,50	0,558 0,557	5,62	6,83	0,41
Salt-soluble fractions	136,30 142,25	1,710 1,658	5,62	8,78	0,53
Stroma fractions	186,80 171,20	0,675 0,679	5,62	3,90	0,23

Based on the data given in Table 4, one can conclude that the most content of the total protein in the test samples makes: 21.97 g/100 g in whole blood, 18.46 g/100 g in low-fat beef, including 6.83 g/100 g in the water-soluble fraction, 8.78 g/100 g in the salt-soluble fraction, 3.90 g/100 g in the stroma fraction.

Since literary sources give no information about which fraction can contain prion proteins, an electrophoretic investigation in polyacrylamide gel using sodium dodecylsulfate was carried out with every fraction of animal proteins of a beef carcass, separated in accordance with the standard procedure presented in the course book of “Meat and Meat Products Investigation Methods”.

DISCUSSION

The PCR method allowed to determine the reaction kinetics and, based on the information received, to judge about the presence and the original amount of the DNA-target in the sample. Besides, the real-time PCR method allows to determine the known mutations in the DNA sequence and their percentage. An important advantage of the method is that there is no need in post-reaction manipulations with the samples and, as a consequence, decrease of contamination risks, reduction of the analysis time and simplification of PCR laboratory organization.

The following items are necessary to carry out the specific reaction:

1) matrix DNA-target;
2) two artificially synthesized primers – oligonucleotide sequences 15-30 bps long;
3) thermoresistant DNA-polymerase (Thermus aquaticus, or Taq DNA-polymerase is used more frequently) remaining active at 94°C and higher;
4) four desoxyribonucleotides.

To choose the DNA-target, a comparative analysis and equalizing of nucleotide sequences of the prion protein PRNP gene, accumulated in GenBank by the present time were carried out. For this purpose, a research through the database of complete nucleotide sequences corresponding to the prion protein PRNP gene was held.

The executed phylogenic analysis confirmed that the sequences of prion protein gene are highly conservative and differ only with conformation and proteolysis resistance connected with it. That is why it is impossible to choose a DNA-target from the prion sequences for the subsequent analysis with polymerase chain reaction.

That is why a kind of PCR has been chosen in this case, which is a real time immuno-PCR, to detect infectious prion proteins, where the DNA molecule is used as a marker. Immuno-PCR allows detection of the pathogenic prion protein using specific antibodies marked by a double-stranded DNA.

CONCLUSION

The executed phylogenic analysis confirmed that the sequences of prion protein gene are highly conservative and differ only with conformation and proteolysis resistance connected with it. That is why it is impossible to choose a DNA-target from the prion sequences for the subsequent analysis with PCR.

A real time immuno-PCR method has been chosen, to detect infectious prion proteins, where the DNA molecule is used as a marker.

A mouse monoclonal antibody 15B3 received with the help of three different sequences (of an epitope) of the human PrP peptide: 15b3-1 includes remnants of 142-148 GSDYEDR(YY); 15b3-2 includes remnants of 162-170 YYRPVDQYS; 15b3-3 includes remnants of 214-226 CITQYQRESQAYY.

It is shown experimentally that 15B3 reacts with PrP^{Sc} of a man, cattle, sheep, deer, mouse and hamster, but does not react with normal PrP^{C} prions. That is why 15B3 has been chosen as an object for further investigations.

BIBLIOGRAPHY

[1] L.V. Borisov. Medical Microbiology, Virology, Immunology. – Moscow, 2001.
[2] V.B. Grigoryev. Prion Diseases of Man and Animals. – Virology Questions, vol. 49 (No. 5), p. 4-12, 2004 (review)
[3] V.A. Zuyev, I.A. Zavalishin, V.M. Roykhel. Prion Diseases of Man and Animals: Doctors Guidance. – Moscow, 1999.
[4] I.S. Shkundina, M.D. Ter-Avanesyan. Prions. Biological Chemistry Successes, vol. 46, 2006 (review)
[5] V.I. Pokrovsky, O.I. Kiselev. Molecular Underpinnings of Prion Diseases. // RAMS Bulletin. – 1998. – No. 10.
[6] V.I. Pokrovsky, O.I. Kiselev, B.L. Cherkassky. – Prions and Prion Diseases. – RAMS, 2004, 384 pages, ISBN 5-7901-0038-4
[7] G. Shlegel. General Microbiology. : translation from German / G. Shlegel. – Moscow: Mir, 1987. – 567 p. 273.
[8] G.Ye. Shultz. Proteins Structural Organization Primciples / G.Ye. Shultz, R.H. Shirmer. - Moscow: Mir, 1982. – 354 pages.
[9] Ing. Jaroslav Petr, DrSc. Phony a ustni dutina. Progresdent, 2004, № 2, s. 12-16
[10] Prion biology and diseases, edited by S.B.Prusiner, Cold Spring Harbor, NY, 1999, ISBN 0-87969-547-1