Format for ANSWERING REVIEWERS

May 4, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: edited manuscript_Techniques to elucidate the mysterious conformation of prions).

Title: Techniques to Elucidate the Conformation of Prions

Author: Martin L. Daus

Name of Journal: *World Journal of Biological Chemistry*

ESPS Manuscript NO: 17542

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

I want to thank you and the reviewers for their comments that will help to improve the manuscript. A point-by-point response to comments of the reviewers is listed below. Additional notes to the original manuscript are highlighted in red. Page numbers are referred to the original manuscript.

Reviewer 1

Comment 1: Background of classification of different strains of prions should be provided in the first paragraph since it will help to understand different tech.

Response 1: Background information about different strains is now provided. The following sentence has been added to the manuscript (page 5) *…to discriminate different prion strains [11,21,22]. In prion research, strains are defined as prion- isolates that, after inoculation into distinct hosts, cause disease with consistent characteristics, such as incubation period, distinct patterns of PrP^{Sc} distribution and spongiosis in the brain. A concrete example…*

Comment 2: Most recent solid review(s) should be used to support the first sentence in the introduction.

Response 2: Recent solid reviews have been added to the references to support the notion that prions are infectious proteins that cause fatal neurodegenerative diseases.
Prion diseases (Johnson; Lancet Neurol.); The prion diseases (Prusiner; Brain Pathol.)

Comment 3: The rationale of applying micro-FTIR in screening and differentiating prion strains are missed, i.e., why small or micro sample can help or improve identification of prion strains is missed**.**

Response 3: The following sentence has been added to the manuscript (page 5). *…can be applied*. *The use of small amounts of sample material allows the preparation of multiple samples within a short time. Spectra from CWD…*

Comment 4: Weakness or limitation of presented techniques should be discussed.

Response 4: Weakness or limitation of presented techniques are discussed in the relevant sections of the manuscript. For example: While FT-IR gives structural information mainly of the secondary structure of proteins other techniques as NMR can reveal higher resolution 3D structural data. The combination of biochemical and biophysical data is important. The following comment has been added to the *Conclusions (page 6) …more structural data* from biochemical and biophysical experiments are required for PrP^{SC}…. *Therapeutic strategies.* A figure (Figure 1 and figure legend) has been added to the manuscript that highlights the presented techniques.

Comment 5: Carefully editing is needed. For examples, the 2nd sentence in the 1st paragraph of introduction is unclear. It might have missed a "which". The 4th sentence in this paragraph does not make since. What are these models if there is no 3D structure? The word of "following" in the 1st sentence of the 2nd paragraph in the introduction may be replaced with "current" or "present". "CD spectroscopy" should have full spelling of CD. The 3rd sentence on page 5 is confusing. Both "occurring" and "that" define diseases and thus should be in the same grammar form, i.e., either -ing or that, and connected by "and". “Further techniques …” on page 5 should be “Other techniques …”.

Response 5: The paper has been edited as suggested by this reviewer. So far, no 3D structure of the complete PrP^{SC}-molecule is available. A figure (Figure 1) on this issue has been added. Models for the structure of PrP^{SC} are based on computer simulation techniques.
In the 2nd paragraph (page 3) “following” has been replaced by “present”. “CD-spectroscopy” has been replaced by “circular dichroism (*CD*)-spectroscopy” (page 4). In the 3rd sentence on page 5 “that” has been replaced by “and”. On page 5 “Further techniques” has been replaced by “Other techniques”.

Reviewer 2

Comment: This is a very well-written review on the techniques studying the structure of prions.

Response: I want to thank the reviewer for the kind comment.

Reviewer 3

Comment 1: This mini-review is concisely written regarding several techniques to assess the conformation of prions. Some minor points are described below. Page 5, line 5 up 1) The end of the sentence "Limited proteolysis….while the less structured N-terminus is cleaved of[38-43]." The "of " is correct? Is it "off"?

Response 1: On page 5 "cleaved of" has been replaced by "cleaved off".

Comment 2: Page 6, line 8 up The sentence "Prions lack a coding nucleic acid…." is unclear. This means that infectious prions lack any nucleic acids? or that because prions have the same DNA sequence, the detection of misfolded isoforms cannot be done by genetics?

Response 2: It is correct that prions are devoid of any coding nucleic acids. Referring to this, prions are unique pathogens. This is mentioned in the abstract of the manuscript. The cellular and the misfolded isoform of the prion protein originate both from the same nucleic acid. Posttranslational modifications are responsible for misfolding. The mechanism of this misfolding is not completely understood. Therefore the detection of the misfolded isoform cannot be done by genetics. This is described in the introduction of the manuscript. A figure (Figure 1) has been added to the manuscript that summarizes this issue.

Reviewer 4:

Comment 1: It would be helpful to directly compare different techniques, such as FTIR, fluorescent spectroscopy, EPR, NMR, H/D exchange, and limited proteolysis, emphasizing the advantages and disadvantages of each.

Response 1: Advantages and disadvantages of the different analytical techniques are mentioned in the text. As already mentioned above it is important (for structural analysis) to combine data from biochemical and biophysical approaches. The following comment has been added to the *Conclusions* (page6) …more structural data from biochemical and biophysical experiments are required for PrP^{SC}… A figure (Figure 1 and figure legend) has been added to the manuscript that highlights the presented techniques. Legend Figure 1: **Different conformations of the prion protein (PrP).** In mammals and humans both isoforms of PrP (the cellular isoform [PrP^{C}] and the misfolded isoform [PrP^{SC}]) are encoded by the same gene (I). PrP^{C} is shown in green (II). Under specific conditions PrP^{C} can be transferred into a misfolded and then putatively infectious conformation (PrP^{SC}). Prion strains can adopt different conformations (indicated by different symbols and colors). When a misfolded isoform of the prion protein becomes infectious it is characterized by specific features as incubation period, PrP^{SC}-spreading, PrP^{SC}-deposition and a distinct potential of infection. For structural analyses of misfolded prion proteins several methods are available. While FTIR, NMR and X-ray structure analyses provide information about the secondary structure as well as 3D-structural information ("direct"), limited proteolysis, fluorescence spectroscopy, EPR, H/D-exchange, cross-linking or mutational analyses reveal more "indirect" structural information that is focused on specific residues within the protein.

Comment 2: I figure or two illustrating the points the authot is making would help readers.

Response 2: A figure has been added to the manuscript (see Response 1; Figure 1).

Comment 3: Grammar needs to be corrected in many places, e.g., “compared with” should be “compared to”, “FTIR on proteins” should be “FTIR of proteins”, “cleaved of” should be “cleaved off”, etc. Extensive editing, preferably by a native speaker, is needed.

Response 3: The paper has been edited by a native speaker. On page 4 “compared with” has been replaced by “compare to”. On page 5 “cleaved of” has been replaced by “cleaved off”. “FTIR on proteins” (page 4) has been replaced by “FTIR of proteins”.

Reviewer 5:

Comment 1: First, the author mentions therapeutic potential, but gives no hints as to how FT-IR might be applied to the development of anti-prion therapeutics. It is easy to imagine how a technique that is sensitive to changes in protein secondary structure could track development of a pathology that is sparked by changes in protein secondary structure and subsequently how therapeutics might prevent this change. This needs to be briefly addressed in the minireview.

Response 1: The prevention of PrP^{C} to PrP^{SC} misfolding or the reversibility of PrP^{SC} back to PrP^{C} is in the main focus of therapeutic approaches. FTIR cannot directly be applied to the development of anti-prion therapeutics. For the understanding of this misfolding process structural data from different techniques is essential. F-IR is only one of the techniques that can help to achieve this goal. I think that comment 1 of Reviewer 5 is already answered by the two sentences: 1) *Definite structural information about prions is indispensable for the understanding of the conversion process and eventually for the design of new therapeutic strategies* (introduction) and 2) *For a better understanding of the conversion of PrP^{C} to PrP^{SC}, more structural data for PrP^{SC} are required. Therapeutic strategies that aim at the prevention of this misfolding process would benefit from such a progress (Conclusions).* This implies that the combination of FT-IR with other high-resolution techniques is needed for a better understanding of the process of misfolding. The aim of the manuscript is to highlight the need of accumulating structural data that may subsequently be useful for therapy. The following comment has been added to the *Conclusions* (page 6) …more structural data from biochemical and biophysical experiments are required for PrP^{SC}…. A figure (Figure 1 and figure legend) has been added to the manuscript that highlights the presented techniques.

Comment 2: Second, the author doesn’t address how far FTIR can go in terms of making a structural model. There is mention that FT-IR is primarily useful in determining secondary structure changes, but doesn’t mention at all if this is the limit of the technology or if there are methods for advancing structure determination further using FTIR. Of course, a detailed discussion of any such efforts is beyond the scope of a minireview, but it is important to define parameters of what can and cannot be learned by a featured technique.

Response 2: The reviewer is right in saying that FT-IR is primarily useful in determining secondary structure and secondary structural changes of prion proteins. The information obtained from FT-IR can be used for establishing a structural model when combining it with data from other high resolution techniques. Certain techniques as NMR are needed for a reliable 3D-structure. This is now also summarized in Figure 1.

Comment 3: Finally, I am surprised that none of David Eisenberg's work on prions is referenced.

Response 3: The following publication has now been added: "The amyloid state of proteins in human diseases. (Cell)"

3 References and typesetting were corrected

Additionally on page 6 "(vi)" has been replaced by "(iv)".

Thank you again for publishing my manuscript in the *World Journal of Biological Chemistry*.

Sincerely yours,

Dr. Martin L. Daus

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