World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:11, No:12, 2017

Biospiral-Detect to Distinguish PrP Multimers from Monomers

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Abstract : The multimerisation of proteins is a common feature of many cellular processes; however, it could also impair protein functions and/or be associated with the occurrence of diseases. Thus, development of a research tool monitoring the appearance/presence of multimeric protein forms has great importance for a variety of research fields. Such a tool is potentially applicable in the ante-mortem diagnosis of certain conformational diseases, such as transmissible spongiform encephalopathies (TSE) and Alzheimer's disease. These conditions are accompanied by the appearance of aggregated protein multimers, present in low concentrations in various tissues. This detection is particularly relevant for TSE where the handling of tissues derived from affected individuals and of meat products of infected animals have become an enormous health concern. Here we demonstrate the potential of such a multimer detection approach in TSE by developing a facile approach. The Biospiral-Detect system resembles a traditional sandwich ELISA, except that the capturing antibody that is attached to a solid surface and the detecting antibody is directed against the same or overlapping epitopes. As a consequence, the capturing antibody shields the epitope on the captured monomer from reacting with the detecting antibody, therefore monomers are not detected. Thus, MDS is capable of detecting only protein multimers with high specificity. We developed an alternative system as well, where RNA aptamers were employed instead of monoclonal antibodies. In order to minimize degradation, the 3' and 5' ends of the aptamer contained deoxyribonucleotides and phosphorothioate linkages. When compared the monoclonal antibodies-based system with the aptamers-based one, the former proved to be superior. Thus all subsequent experiments were conducted by employing the Biospiral -Detect modified sandwich ELISA kit. Our approach showed an order of magnitude higher sensitivity toward mulimers than monomers suggesting that this approach may become a valuable diagnostic tool for conformational diseases that are accompanied by multimerization.

Keywords : diagnosis, ELISA, Prion, TSE
Conference Title : ICBEM 2017 : International Conference on Biomedical Engineering and Medicine
Conference Location : Paris, France
Conference Dates : December 28-29, 2017