PHYSICO-CHEMICAL AND BIOLOGICAL CHARACERIZATION OF A MINI-PRION PEPTIDE (PRP90-231)

Corsaro A., 4° anno del corso di Dottorato di Ricerca in Neurofisiologia e Neurofarmacologia ciclo XIV. Durata del corso in anni: 4. Sede di servizio: Sezione di Farmacologia e Neuroscienze, Dipartimento Oncologia Biologia e Genetica Università di Genova.

Prion diseases are characterised by spongiform degeneration of the CNS, neuronal death, amyloid deposition and astrogliosis. During these diseases the prion protein (PrP^{C}) a cellular glycoprotein is converted in an altered isoform (PrP^{Sc}) that accumulates within the brain. Synthetic or recombinant polypeptides are commonly used to elucidate the mechanism of proteins involved in neurodegenerative diseases. Here we describe a procedure, which allows the production, purification and correct refolding of human protein prion fragment 90-231, the core protease resistant of the PrP that accumulates in the brain of the affected subjects. We describe the synthesis of the polypeptides 90-231 of the wild type. Using a gluthatione S-transferase (GST) protein fusion approach, milligram amounts of polypeptides were obtained after expression in *E. coli*. The recovery of the purified fusion protein was monitored following the evaluation of the GST activity. The PrP fragment was released from the fusion protein immobilized on a glutathione-coupled agarose the by direct cleavage with bovine thrombin. The recombinant protein was identified by comassie stained acrylamide gel and by immunoblotting employing a monoclonal anti-PrP antibody. Using a gel filtration chromatography we obtained the purification of the peptide. The peptide was mainly in alpha-helix structure as analysed by circular dichroism and showed an intact disulfide bridge. We analysed the structural changes after partial thermal denaturation. PrP90-231 was incubated for 1 h at different temperature and analysed for the intrinsic fluorescence of a unique tryptophan in position 99. The protein resulted stable for 30 min at 4°C, at higher temperatures (37°, 42°, 53°) a significant and irreversible fluorescence quenching occurred already after short incubation times and the rate of the conformational changes increased with the temperature. By using the Eyring formalism the DELTA-H of denaturation was estimated to be about 20 kJ/mol. These results suggest an irreversible conformational change involving the region surrounding Trp99 likely dependent by protein aggregation. This hypothesis was confirmed by means of Western blot analysis, using the 3F4 monoclonal antibody. Interestingly both the florescence quenching and the protein aggregation (measured by the Western Blot analysis) were completely abolished by the coincubation with reducing agent (1mM DTT), suggesting that aggregation process may be dependent on intermolecular disulphide bonds. We measured the PrP90-231 fibrillogenesis by fluorescence emission of Thioflavin-T. PrP90-231 samples are pre-incubated at 37°C for 24 hrs in the presence or in absence of DTT and confirmed the hypothesis that the fibrillogenesis process is dependent on the formation of disulphide bridges.

Finally we analysed the toxic effects of PrP90-231 on the human neuroblastoma SHSY5Y cells lines using the MTT test. PrP90-231 induces cell death only if it is pre-incubated at 53°C for 1 hrs conversely pre-incubation at lower temperature doesn’t influence cell viability. This data well correlate with the irreversible conformational change induced by incubation at 53 °C of PrP 90-231.

In conclusion recombinant hPrP90-231 shows a high tendency to form macro-aggregates in a temperature-dependent fashion; this aggregates show the characteristics of amyloid fibril. The aggregation process was reverted by the co incubation with reducing agents suggesting that it is dependent on the disulphide formation of bonds; moreover temperature-induced structural change of PrP90-231 produces toxic effects on SHSY5Y cells lines.