Electrochemical determination of PrP and its interactions with metals and metallothionein

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PrPC is glycosylphosphatidylinositol-anchored host glycoprotein normally present in brain (Dormont, 2002)

This protein with predominance of α-helix structure can be converted to an abnormal protease resistant isoform with increased ratio of β-sheet structure called prion (PrPSc) (Kong et al, 2013)

PrPSc isoform can cause a range of slow neurodegenerative disorders called transmissible spongiform encephalopathies (Tiraboschi & Tagliavini, 2013). The most famous prion caused disease is BSE

pRSET B cloning kit (Invitrogen, Germany) for high-level expression of recombinant proteins in E. coli was used. For subsequent E. coli cultivation we followed the manual by Invitrogen

Expression of PrPSc in E. coli was verified by gel electrophoresis and western-blot

Western blot – verification of prion protein presence in harvested cells

- 25% SDS page gradient gel
- 0.1% SDS membrane
- Blocking: 5% BSA 1 hour
- 1:1000 dilution of rabbit polyclonal antibody to prion protein 1 hour
- 1:2000 dilution of HRP (Amersham) 2 hours
- 5 min AEC reaction
• PrP may play a role in cell signaling or in binding and transport of Cu(II) and Zn(II) ions (Gasier-Widen et al., 2005; Kozlowski et al., 2012). Cu ions together with Zn ions are involved in the formation of amyloid plaques in case of neurodegenerative disorders (Pedersen et al., 2012).

According to some authors Cu ions can destabilise the native fold of PrP, and can facilitate the conversion to PrP\(\beta\) isoform (Pouran et al., 2013).

• Metallothionein (MT) fulfills multiple functions including the involvement in zinc and copper homeostasis and protection against heavy metal toxicity and oxidative damage. Due to its physiologic role, zinc and copper belong to the most investigated metal ions connected to metallothionein.

• Brain specific subtype of MT is called MT-III and this protein is able to bind copper when Cu homeostasis is disrupted.
Electrochemical determination of PrP and MT (DPV coupled with adsorptive transfer stripping technique)

- Constant PrP concentration 100 µg/ml and various conc. of Cu and Zn (100, 50, 25, 12.5, 6.25, 3.125 µg/ml) measured in acetate buffer pH 5
- The diagram below shows ascending or descending trends of PrP and metals interactions

PrP = Cu

PrP = Zn

- Measured in sodium phosphate buffer pH 7
- AdTS = accumulation time = 120s
- Electrochemical signal corresponds to the concentration
- Dependence of all peaks is linear (R² higher than 0.9)

PrP + PrP = Zn

- In case of PrP and MT interaction there are two coalesced peaks
- Calibration curves of peaks are linear (R² higher than 0.9)

MT = PrP (constant MT concentration = 8µg/ml)

- There is a massive change of structure

MT = PPrP (constant PrP concentration = 100µg/ml)

- In case of PrP and MT interaction there are two coalesced peaks
- Calibration curves of peaks are linear (R² higher than 0.9)
• Recombinant PrP\textsuperscript{C} was produced, isolated and purified

• PrP\textsuperscript{C} was used for electrochemical determination and for an investigation into its interactions with metals and metallothionein

• Massive interaction was discovered especially in case of MT and PrP\textsuperscript{C} interaction

• We presume that a change of the peak position is caused by the formation of MT tetramers enclosing the PrP\textsuperscript{C} molecule to the center in case of high PrP\textsuperscript{C} concentration

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