L-2 Screening for bioactive compounds from natural products by ELISA assay

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Combinatorial chemistry is one of the most interested topics in the area of drug discovery. One of the most important points is how to find a lead compound that gives the seed structure for designing of a combinatorial library. Natural products is suitable for searching a new bioactive compound with new structure. We have carried out systematic screening works to find natural products possessing the effects on inter- and intra-cellular signaling. Two hundreds extracts of medical plants and two thousands microbial culture broth samples have been tested for the induction and inhibition of IL-2 or IL-6 production (Fig. 1). ELISA is an efficient method for screenings from such a large number of samples. Now, we apply this method to search prion-binding agents.

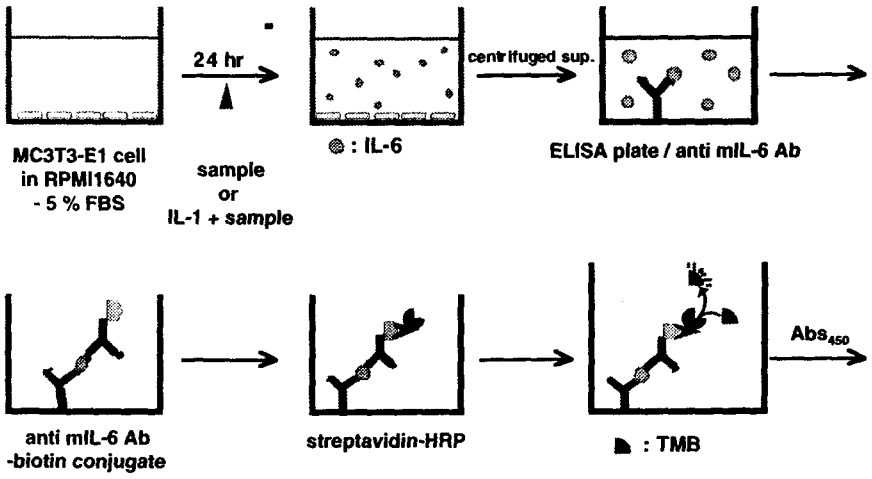
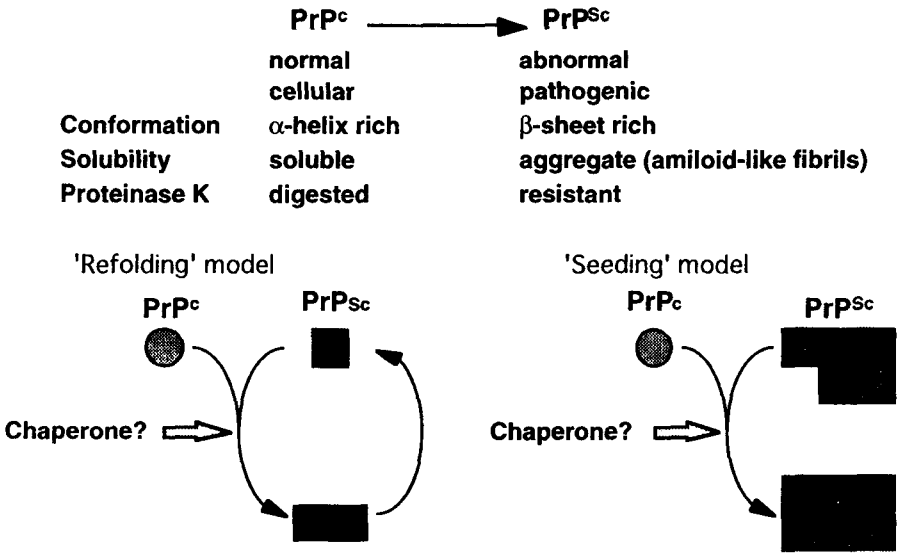


Fig. 1 Detection of IL-6 production by ELISA

Prions is known as the proteinaceous infectious agents that cause transmissible spongiform encephalopathies, such as scrapie and bovine spongiform encephalopathy (BSE) in animal, or Kuru and Creutzfeldt-Jakob disease (CJD) in human. Stanley Prusiner was awarded the Novel prize in Physiology or Medicine by the 'protein-only' hypothesis. Prion proteins are host-encoded and hold at least two conformations. One is PrP^{c}, that is present in all mammals and expressed on the surface neurons. Another one is PrP^{Sc}, that is pathogenic and partial resistant for proteinase K digestion, and forms insoluble β-amiloid like fibrils. Research projects to investigate normal function of PrP^{c} are now undergoing by many groups, but it has not been revealed for the present. PrP^{c} and PrP^{Sc} have the same chemical properties, but are different in secondary and tertiary structures. PrP^{c} folds an α-helix rich conformation, while PrP^{Sc} is β-sheet rich. PrP^{Sc} causes the conversion of PrP^{c} into PrP^{Sc}. The ability for replication in a sense is the most characteristic property of prions and enables the infection of prion diseases. The mechanism of the conversion has not been completely revealed, but the other factor, 'protein X', has known as a necessary factor for the conversion.

Fig. 2 Prion hypothesis

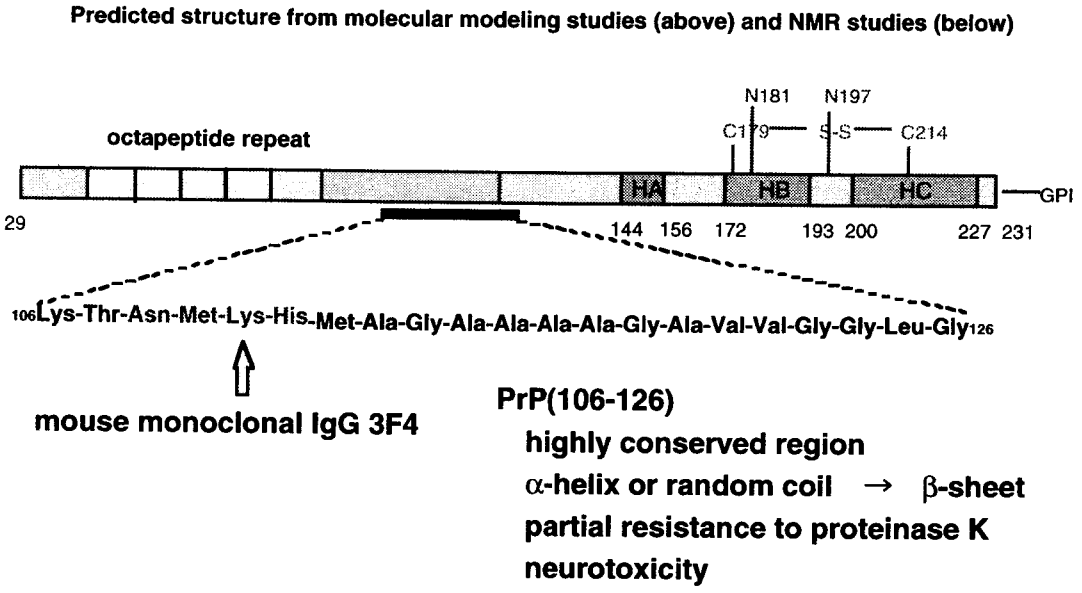


We notice the point, that interactions between PrP^{Sc} and PrP^{c} or some other proteins are necessary for the conformational change of prions, and proposed a new approach to prion studies from a viewpoint of molecular interactions. Prion-binding agents are expected to show inhibitory or stimulating effects on the conversion of prions, and be applied as biochemical tools or seed compounds for development of anti-prion drugs. So, we set up a ELISA system to detect prion-binding activities of samples, and tested extracts of medical plants and microbial culture broth samples.

Prion protein can be divide into two regions, hydrophilic N-terminal and hydrophobic C-terminal regions. It has two glycosylation sites and one disulfide bonding site in C-terminal region, and a characteristic repeating sequence of octapeptide in N-terminal region. Normal prion protein is anchored to the cell membrane by a glycophosphatidyl inositol (GPI) anchor linked to the C-terminus (Fig. 3). Recently, the NMR-derived structure of PrP^{c} has been established. The NOE data show that C-terminal region folds a globular structure containing three α-helix, while N-terminal region is unstructured and highly flexible.

Whole prion protein was not available except for some special situations, so we decided to use a fragment peptide of prion protein, and selected PrP(106-126) for the target of binding. This peptide is 21-mer length peptide from prion protein, located the boundary region between hydrophobic C-terminal and hydrophilic N-terminal regions. The boundary region is one of the highest conserved regions through mammal species in primary structure of prion. PrP(106-126) is the shortest peptide that maintains (1) the ability of conversion of α-helix into β-sheet, (2) partial resistance to proteinase K digestion and (3) neurotoxicity, but no infectivity of prion disease has been detected at the present time. Therefore, this peptide is an useful model for conformational analysis of prion protein. Furthermore, a monoclonal antibody recognizing the N-terminal region of PrP(106-126), 3F4, is commercially available (Fig. 3).

Fig. 3 Prion peptide (106-126)



We have constructed ELISA assay system with the peptide PrP(106-126) and antibody 3F4 to detect inhibition of binding between prion and the antibody. This system can not cover all of the bindings to prion protein and can not exclude some non-specific bindings. It is necessary to check the selectivity of the observed bindings by the other methods. However, this system is useful for the primary screening to reduce the candidates from a huge number of samples, and is the first case of screening work targeting prion proteins from natural products, as far as we follows. In this seminar, the results of the screening and attempts to investigate the binding selectivity will be discussed.