利用普利昂蛋白 E196 單點突變探討電荷對普利昂聚集的影響

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普利昂疾病，又稱為傳染性海棉腦病（TSE），是一種致命的神經退化性疾病。人類的普利昂疾病有庫賈氏症 (CJD)、致命的家族性失眠症 (FFI)等，在動物方面有羊搔症、狂牛症。致病性的普利昂蛋白富有穩定的β折板結構，在腦部產生堆積後形成斑塊，導致神經細胞死亡。有研究顯示，普利昂蛋白 E196 的突變會產生結構的改變；相對於原生型態，E196K 的結構會較於鬆散，且在纖維核心的部分會由疏水腔變為親水腔。本研究團隊之前的研究結果顯示槲皮素與普利昂蛋白的 E196 緊密結合後可改變其纖維化的特性。原生型普利昂蛋白中，E196 帶負電荷。為探討殘基 196 之電荷效應，我們將 E196 分別突變為帶正電荷的 E196K 與電中性之 E196A，以探討電荷對結構及蛋白質特性的影響，進而檢視槲皮素與這三種電荷狀況之普利昂纖維的作用狀況，以利後續的藥物開發。我們利用了 ThT 螢光染劑檢測普利昂纖維化的狀況、以圓二色光譜儀檢視普利昂蛋白質的結構、螢光顯微鏡及穿透式電子顯微鏡觀察普利昂纖維之構型。

黃酮類化合物是一類多酚類物質，廣泛分佈於植物中，具有很強的生物活性，包括抗氧化、抗癌、抗炎等作用，可預防神經退行性疾病。本研究團隊已得知槲皮素有抑制普利昂蛋白聚集、分解普利昂纖維的效果。進一步地，將陸續測試盧丁、橙皮素、橙皮苷等黃酮類化合物對普利昂纖維的分解效果。

Study on the Effect of Electric Charge on Prion Aggregation Using a Single Point Mutation of Prion Protein E196

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Prion diseases, also called transmissible spongiform encephalopathy (TSE), are a group of fatal neurodegenerative diseases, including Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI) in human and Scrapie, bovine spongiform encephalopathy (BSE) in animals. Pathogenic prion proteins are rich of stable β-sheets. Their accumulation in the brain forms amyloid plaques leading to nerve cell death. Many researchers have found that single point mutation on E196 changes the structure of prion. Compared with the wild-type, the structure of E196K is looser, and the hydrophobic cavity of the fibril core turns hydrophilic. Our previous research showed that quercetin can change its fibrillization properties by tightly binding to E196 of prion protein. In the native prion protein, E196 is negatively charged, while E196K mutant is positively charged, and E196A mutant is electrically neutral. We use different mutants to investigate the effect of charge on structure and protein aggregation properties, and then examined the interaction of quercetin with prion fibrils in these three charged states for the following development of the drugs. We have detected the fibrillization of prion by thioflavin T fluorescence, the secondary structure of prion by circular dichroism spectroscopy, and the morphology of prion fibrils by fluorescence and TEM imaging.

Flavonoids are a class of polyphenols that are widely distributed in plants and have strong biological activities, including antioxidant, anti-cancer, anti-inflammatory effects, and can prevent neurodegenerative diseases. Our group has found that quercetin can inhibit fibrillization of prion and can disaggregate prion fibrils. Furthermore, we will test the disaggregation effect of rutin, hesperidin and hesperitin. .