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3aF05

プリオンのダイナミクスに基づく抗プリオン薬のデザイン

○桑田 一夫[1]、鎌足 雄司[1]、松本 友治[1]、中村 寛則[1]、早野 陽介[1]
[1]岐阜大・CEID

Dynamics based drug design for prion diseases

Kazuo Kuwata, Yuji O. Kamatari, Tomoharu Matsumoto, Hironori K. Nakamura, Yousuke Hayano. (Center For Emerging Infectious Diseases, Gifu University)

Prion proteins are key molecules in transmissible spongiform encephalopathies (TSEs), but the precise mechanism of the conversion from the cellular form (PrPC) to the scrapie form (PrPSc) is still unknown. We previously uncovered slowly fluctuating residues in PrPC distributed diffusely at B & C helices using high pressure NMR and CPMG relaxation dispersion measurements. Applying a dynamics-based drug design (DBDD) strategy, we discovered a compound GN8 efficiently reduced PrPSc in a TSE-infected cell culture model. Subsequently, administration of GN8 was found to prolong the survival of TSE-infected mice. Heteronuclear NMR showed that the critical binding sites include A-S2 loop (N159), helix B (V189, T192 and K194) and B-C loop, indicating that the intercalation between the A-S2 loop and the B-C loop hampers the pathogenic conversion process. Our results demonstrate that the pathogenic conversion is a rare event emanated from the slow conformational fluctuation occurring around the hot spots in PrPC. Furthermore, DBDD focusing on the hot spots of PrPc will open the way to the development of novel anti-prion drugs.

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3aF06

NDSBによる蛋白質の凝集防止とNMR測定への応用

向 瓏[1]、石井 毅[1]、細田 和男[1]、井上 裕介[1]、行木 信一[1]、窪田 健二[1]、楯 真一[2]、河野 俊之[3]、○若松 馨[1]
[1]群馬大院・工学系・化学生物、[2]広島大院・理学系・数理分子、[3]三菱化学生命研

Prevention of protein aggregation by NDSB and its application to NMR measurements

Long Xiang (1), Takeshi Ishii (1), Kazuo Hosoda (1), Yusuke Inoue (1), Nobukazu Nameki (1), Kenji Kubota (1), Shin-ichi Tate (2), Toshiyuki Kohno (3), and Kaori Wakamatsu (1) (1: Dept Chemistry & Chemical Biology, Graduate School of Engineering, Gunma Univ; 2: Dept Mathematical & Life Sciences, Graduate School of Science, Hiroshima Univ; 3: Mitsubishi Kagaku Institute of Life Sciences)

In solution NMR, better sensitivity and resolution are obtained by raising sample temperature, but proteins aggregate at a too high temperature due to thermal denaturation. Because pharmaceutically important proteins tend to aggregate more easily than housekeeping proteins, prevention of protein aggregation is one of challenging tasks in protein NMR. Here we report the application of NDSB (non-detergent sulphobetaine) for protein NMR. NDSB enables acquisition of protein NMR spectra at higher temperatures by stabilizing proteins. Sample precipitation and time-dependent decrease in NMR signals due to higher temperature were prevented by supplementing NDSB in several protein samples. This enabled unambiguous assignment of several signals in a 40-kDa protein without deuteration by triple-resonance spectroscopy. The mechanism whereby NDSB prevents protein aggregation will also be discussed.

3P062

両親媒性ポリマーによるケミカルシャペロン活性の向上

○冨田 峻介[1]、浜田 寛之[1]、白木 賢太郎[1]
[1]筑波大院・数理物質

Enhancement of chemical chaperon activity by amphiphilic polymers.

Shunsuke Tomita (1), Hiroyuki Hamada (1) and Kentaro Shiraki (1). (1: Inst. Appl. Phys., Univ. Tsukuba)

Aggregation is a serious problem for biotechnology and pharmaceutical application of proteins. Although various techniques have been developed to control protein aggregation, one of the versatile approaches is the addition of small molecules, typically arginine (Arg), as a chemical chaperon. We investigated thermal aggregation and inactivation of lysozyme in the presence of mixed solution that contains small molecules and polymers. Almost all of lysozyme formed inactive aggregates by heat treatment (98 °C 10 min) even in the presence of single component of aggregation suppressors ,such as Arg and NH4Cl, or amphiphilic polymers, such as poly(ethylene glycol)(PEG) or poly(vinyl pyrrolidone), while the heat-induced aggregation and inactivation of lysozyme was dramatically suppressed in the presence of mixed component of both aggregation suppressors and polymers. The enhancement of the chaperon activity has not shown in the presence of hydrophilic polymers, such as PEG 200, poly(vinyl alcohol), poly(acrylic acid), dextran, and Ficoll 70. The thermally unfolded protein exposing hydrophobic surface is prone to form aggregates. The hydrophobicity of amphiphilic polymers may stabilize the labile molecules by adsorbing to the weak hydrophobic surface. This result provides a new insight into the protein folding with chemical chaperon mediated by compatible polymers.

3P063

界面活性剤混合系におけるタンパク質の二次構造変化

○森山 佳子[1]、RAZALI AZAIMA[1]、竹田 邦雄[1]
[1]岡山理大工

Secondary Structural Change of Protein in Mixed System of two Surfactants

Dep. Applied Chemistry and Biotechnology, Okayama Univ. of Science

It was found that the structural change of protein in the mixed system of two surfactants depends on the additive order of the surfactants. As a protein, bovine serum albumin (BSA) was used. This protein has been most frequently adopted in the studies of the interactions with ionic surfactants. The zwitterionic N-tetradecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate (SB3-14) was used to prepare the mixed solutions with anionic sodium dodecyl sulfate (SDS) or cationic dodecyltrimethylammonium bromide (DTAB). The circular dichroism measurements were carried out at 25 °C to examine the secondary structure of BSA in the mixed system of ionic and zwitterionic surfactants. The helicity of 66 % for BSA decreased down to 50 % in solutions of SDS or DTAB alone, while it decreased only to 55 % in solution of SB3-14 alone. In the case of mixture of SDS and SB3-14, the late addition of SB3-14 reformed the helical structure of BSA disrupted by SDS. This reformation required higher SB3-14 concentrations as the initial SDS concentration increased. The helical structure of the protein, which was firstly disrupted by SB3-14, was reformed by the addition of slight amounts of SDS. In the case of mixture of SDS and SB3-14, the late addition of one surfactant tended to reform the helical structure disrupted by the other. However, such a tendency was not observed in the case of mixture of DTAB and SB3-14. The late addition of SB3-14 accelerated to disrupt the helical structure in DTAB solutions of low concentrations.