2P044 位置特異的同位体ラベルによるアミロイドβ集積体の高次構造解析
○吉田 研誠^{1,2}, 奈良 雅之³, 小中原 猛雄², 岡田 知子¹, 森井 尚之¹
¹産総研・生物機能工学・分子認識, ²東京理科大・理工学・工業化学, ³東京医科歯科大・教養部・化学
Structural analysis on self-assembled amyloid-β by specific isotope labeling
Kensei Yoshida(1)(2), Masayuki Nara(3), Takeo Konakahara(2), Tomoko Okada(1) and Hisayuki Morii(1). (1: Natl Inst Adv Industrial Science and Technology (AIST); 2: Faculty of Science and Technology, Tokyo Univ of Science (RIKADAI); 3: Dept of Chemistry, Tokyo Medical and Dental Univ (TMDU))

Alzheimer's disease is most likely to be brought about by the aggregation of amyloid-β(Aβ) peptides. In order to reveal the fine structure in its amyloid fibrils, a series of isotope-labeled Aβ peptides were prepared by introducing a special amino acid with nitrogen-15 atom at its α-amino group into specific sites. The amyloids were analyzed by ATR method of infrared spectroscopy. The non-labeled amyloid of (21-42) fragment exhibited absorption bands at 1625 and 1653 cm⁻¹ assigned to β-structure (57%) and random coil structure (43%), respectively. ¹⁵N-labeled amyloids at the positions of A30, G33, L34, V36, and G37 exhibited enhanced absorptions at 1685 cm⁻¹, which correspond to antiparallel β-structure. On the other hand, ¹⁵N-labeled amyloids at the positions of V24, V39, V40, and A42 exhibited similar spectra to the non-labeled amyloid. These results suggest that the region (30-37) of Aβ(21-42) is likely to form a stem of amyloid fibril bearing cross-β structure. On the basis of our proposing model for general amyloid structures, the inter-strand interaction between hydrophobic I31 and V36, and that between M35 and I32 as well, seem to be responsible for the formation of the stem. It is noteworthy that C-terminal region (40-42), which is reported to bring about the different behaviors in aggregation between Aβ(1-42) and (1-40), is random coil structure for not only Aβ(21-42) but also Aβ(1-42). Further analysis on Aβ(1-42) is in progress.

2P046 時間分割蛍光法によるRNase T1 中 Tryptophan 残基の複数の誘電緩和特性に関する研究
2aE11 ○乙須 拓洋¹, 西本 悦子¹, 宮崎 義史¹, 山下 昭二¹
¹九大院・生資環
Time-resolved fluorescence study on multiple dielectric relaxation properties of single tryptophan residue in RNase T1
Takuhiro Otosu, Eisuko Nishimoto, Yoshifumi Miyazaki, Shoji Yamashita (Institute of Biophysics, Faculty of Agriculture, Kyushu University)

Time-resolved fluorescence spectroscopy has screened out the intra- and intermolecular interaction mechanism on the energy relaxation process. Fluorescence decay kinetics of protein is usually described as linear combination of exponentials, even in single tryptophan (Trp) proteins. Rationalizing this peculiar kinetics, we can elucidate the function-structure relationships of protein by time-resolved fluorescence spectroscopy. But the origin of that multiexponentiality is in controversy and this hampers the interpretation of fluorescence data. Among several interpretations, dielectric relaxation process during excited state is a potent factor in multiexponential decay kinetics of Trp residue in proteins. Our dielectric relaxation study on calmodulin-melittin interaction confirmed the validity of dielectric relaxation model to explain the multiexponentiality of Trp fluorescence in protein. Furthermore, we found multiple distinct dielectric relaxation properties in single Trp of RNase T1. This result suggested that Trp in RNase T1 has several different conformations and/or Trp is surrounded by different electric field. In this conference, we make a presentation about precise examination of this peculiar dielectric relaxation property of Trp in RNase T1 by time-resolved fluorescence spectroscopy, and we also discuss about interaction with guanosine monophosphate, RNase T1 inhibitor.

2P045 口腔連鎖球菌グルコシルトランスフェラーゼ-I によるデキストラン結合の熱力学的解析: ストイキオメトリーに対する触媒ドメインの影響
2aE10 ○小松 英幸¹
¹九州工大
Thermodynamics of the dextran binding of mutans streptococcus glucosyltransferase-I: Effect of the catalytic domain on the stoichiometry
Hideyuki Komatsu (Dept Bioscience & Bioinformatics, Kyushu Inst of Technology)

Glucosyltransferase-I (GTF-I) from mutans streptococcus catalyzes the transfer of a glucose unit from sucrose to a dextran chain.
Suc + (Glc)_n → Fru + (Glc)_{n+1}
GTF-I (175 kDa) is composed of an N-terminal catalytic domain (GSd, 110 kDa, GS for glucansucrase) and a C-terminal glucan-binding domain (GBd, 65 kDa). GBd itself strongly binds to dextran, and characteristically contains six homologous, directly repeating units. Recently, we studied the binding of a series of C-terminal truncated GBds to dextran by isothermal titration calorimetry (ITC) (Komatsu *et al.*, 2007). The thermodynamic parameters (i.e., the number of glucose units constituting the dextran stretch bound to the protein (*n*), the enthalpy change (ΔH), and the Gibbs energy change (ΔG)) were proportional to the molecular mass of the GBd truncate, which result suggests that identical glucose-binding subsites are arranged consecutively from the GBd N-terminus.
In this study, to investigate the effect of GSd on dextran binding of GBd, the dextran binding of a series of C-terminal truncated GTF-Is have been analyzed by ITC. The values of ΔH and the ΔG of GTF-I truncates were similar to those of a series of the GBd truncates, and proportional to the molecular mass of the truncate. However, the values of *n* were about 30 units of glucose corresponding to that of full-length GBd, and constant in the all truncates. These results suggest that GBd in GTF-I truncates binds to dextran likewise GBd alone and that GSd keeps the intervals between GBd-binding sites on dextran chain.

2P047 抗シグトキシン抗体 10C9 Fab—シグトキシン間相互作用の構造学的・熱力学的解析
○宇井 美穂子¹, 田中 良和¹, 円谷 健², 藤井 郁雄², 井上 将行^{3,4}, 平間 正博³, 津本 浩平¹
¹東京大院・新領域・メディカル, ²大阪府立院・理, ³東北大院・理, ⁴東京大院・薬
The structural and thermodynamic analysis of the interaction between ciguatoxin and its Antibody 10C9 Fab
Mihoko Ui(1), Yoshikazu Tanaka(1), Tsumuraya Takeshi(2), Ikuro Fujii(2), Masayuki Inoue (3, 4), Masahiro Hirama(3), Kouhei Tsumoto(1). (1: Graduate School of Frontier Sciences, The University of Tokyo; 2: Graduate School of Science, Osaka Prefecture University; 3: Graduate School of Science, Tohoku University; 4: Graduate School of Pharmaceutical Sciences, The University of Tokyo)

A ciguatoxin (CTX3C) is a rod-like poly-ether compound, which is one of the marine toxins causing a human intoxication. To understand the recognition mechanism of CTX3C by a therapeutic antibody 10C9, we determined the crystal structure of 10C9 Fab in uncomplexed form and in complex with each fragment of CTX3C, CTX3C-ABCD and CTX3C-ABCDE, at a resolution of 2.6 Å, 2.4 Å and 2.3 Å, respectively. It was revealed that the Fab had an extraordinary large and deep pocket constructed by the interface between VH and VL to accommodate CTX3C, and that both antigens were inserted into the pocket longitudinally. The antigen-antibody complex structures have suggested that the Fab has changed its structure upon complexation. DSC analysis has suggested the quite stable thermodynamic stability of CTX3C-ABCDE-10C9 Fab complex from that of CRX3C-ABCD. ITC analysis has shown that the Fab discriminated between CTX3C-ABCD and CTX3C-ABCDE by the difference of stability of the two complexes themselves, with association constants of 5.4x10⁶ M⁻¹, 9.0x10⁷ M⁻¹, respectively. Besides some hydrogen-bonds and van der Waals interactions at the binding pocket, some CDR loops seem to be stabilized upon complexation, perhaps due to recognition of the antigen at the bottom of the flexible loops. The antigen recognition mechanism of the 10C9 Fab provides a template for understanding how proteins recognize not only a rod-like poly-ether compound such as CTX3C but a large and rigid chemical compound which cannot adjust its structure to the targets.