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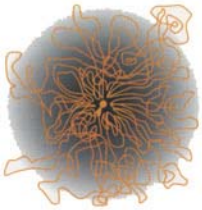
ABSTRACTS



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[Gregor Johann MENDEL] by Kiyoshi FUKUSHIMA

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4P-B-079 Thiol-maleimide chemistry based extension arm facilitated PEGylation of proteins

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The thiol-maleimide chemistry based PEGylation that we developed to generate non-hypercoagulative hexaPEGylated Hb increases the accessibility of protein amino groups to PEG reagents by introducing extension arms on them and still retaining their positive charge. We have now developed a new PEGylation platform that neutralizes the charge of amino groups and adds extension arms of desired length using a reagent of the general structure X-alkyl-S-S-Py (X = succinimidyl or sulfosuccinimidyl, alkyl chain = variable length extension arm, S-Py = thiopyridyl). The thiol group at the distal end of the extension arm is protected as a mixed disulfide of thiopyridine. The thiol group is regenerated for the conjugation of PEG-maleimide once the extension arm is linked to protein through an isopeptide linkage. PEGylation of Hb using N-Succinimidyl-3-(2-PyridylDithio)-Propionate adds an extension arm of 6.8 A° and is used to generate a new hexaPEGylated Hb. PEGylation of RBC using Sulfosuccinimidyl-6-(3' -(2-PyridylDithio)-Propionamido)-Hexanoate added an extension arm of 15.6 A° and masked the D antigen more efficiently than the direct PEGylation without adding extension arm. An extension arm of 9.4 A° with a maleimide moiety at its distal end is added on human serum albumin (HSA) amino groups with N-[e-Maleimidocaproyloxy]Sulfosuccinimide ester. The maleimide moieties are modified by thioPEG, forming a hexaPEGylated HSA. The extension arm facilitated PEGylation is expected to facilitate the generation of PEGylated protein therapeutics with multiple copies of PEG-chains on protein molecule.

4P-B-080 Hypochromic Effect of Prion N-Terminal Octapeptide Repeat by UV Absorption Spectroscopy

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The neurodegenerative diseases like bovine spongiform encephalopathies (BSE) are caused by the confomation change of the C-terminal α-helical domain of prion protein (PrP^C) into its β-sheet-rich abnormal isoform. Prion's N-terminal domain consists of a sequentially repeated region, in which octapeptide GQPH-GGGW is connected in tandem 4 times. Acknowledging the periodic presence of His and Trp in this octarepeat region, we assumed the π-π stacking interactions between prion molecules via this repeat structure. We have demonstrated the presence of such interactions for the octarepeat peptides or PrP^C N-terminal domain on a MALDI-TOF mass spectroscopy. In the present study, we measured their UV spectra to analyze the hypochromic effects expected from the π-π stacking interactions. Analogues of octarepeat peptides were prepared as acetyl amides, and those include 24-mer peptide Ac-(GQPHGGW)$_3$-NH$_2$ (OP3) and 32-mer peptide Ac-(GQPHGGGW)$_4$-NH$_2$ (OP4). All the UV spectra were measured for the aqueous solution with the same peptide concentrations. When the molar extinction coefficients were caluculated using the absorbance at 280 nm, a distinct reduction was obserbed in proportion to the numbers of repeats, or the Trp residues. This hypochromic effect was diminished clearly in lower concentrations of OP3 and OP4. These results indicated that the intermolecular hydrophobic interactions of Trp residues between the octarepeat peptides are dependent upon the number of repeats.

4P-B-081 Ligand predilection, classification and structure of the WW domain

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The WW domain is a protein module that mediates protein-to-protein interactions by binding to proline-containing ligands, and plays important roles in signal transduction, cell cycle control, transcriptional regulation and other divergent cellular events. Previously, the WW domains were classified into at least four groups (I - IV), based on their binding specificity. In order to examine it, we carry out exhaustive ligand-binding experiments of the WW domains with surface plasmon resonance, and find out that the specificity of the WW domains are classified into three major groups, because the specificity of Groups-II and -III is so similar. We carry out structural analyses of the WW domains with homology modeling and nuclear magnetic resonance, leading to the observation that these three groups have their own structural characteristics on their surface, such as the Tyr groove, XP2 groove and p patch. As a result, we propose that Groups-II and -III are merged into a large group, Group-II/III, which recognizes Pro-rich sequences with the XP2 groove. The Pro-rich sequence is the most frequent sequence in eucaryotic genome, which implies or supports diverse importance of the Group-II/III WW domains in cellular processes and diseases, such as the regulation of transcription, cytoskeleton, flowering and neurodegenerative diseases.

4P-B-082 Human FAMSBASE, protein structural model database, with high frequency update

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he large number of genes on human chromosomes has been clarified. The 4 amino acid sequence database, hsap0, hsap1, hsap2 and huge0, was found in GTOP database(http://spock.genes.nig.ac.jp/~genome/gtop-j.html).And they can be available through the Internet, and totaly there are 102 thousands.In this research, the full automatic protein structure homology modeling method program, "FAMS", was used to calculate structural models about all the human genes that can come to hand, and the relational database was constructed. Its database was named "human gene FAMSBASE".For the creation of the database we used the same technique as the "famd" team which participated in the international contest "CASP6", and, at that time, a statistics processing was carried out as the index GDT_TS showed a high value. In an international contest "CASP6", an important index is GDT_TS which shows the degree of distance coincidence of modelled C-alpha atom in the amino acid residues compared with the experimental structures. Positioning of the "famd" team in the contest was evaluated better, about not only GDT_TS but also various indices, such as RMSD, hydrogen bonds, short contacts and dihedral angles of main chain and side chain. And it expresses worth of this database well.Then the database is updated automatically every week while PDB update.

4P-B-083 Thermodynamic Molecular Switch Controls Chemical Equilibrium in Interacting Biological Systems

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The Planck-Benzinger method which we have applied to a wide variety of interacting biological systems provides a means of determining the innate temperature-invariant enthalpy, $\Delta H°(T_\theta)$, thermal agitation energy, or the heat capacity integrals, and allows precise determination of (T_{Cp}), (T_h), (T_s) and (T_m). Our studies have demonstrated that biological interactions will always exhibit negative value of the Gibbs free energy change at a well-defined temperature, (T_s), which is the thermal set point. The critical factor in this thermodynamic molecular switch is a change of a sign in $\Delta C_p°(T)_{reaction}$ which determines the behavior patterns of the Gibbs free energy change, and hence a change in the equilibrium constant, K_{eq}, and /or spontaneity. The subsequent, mathematically predictable changes in $\Delta H°(T)$, $T\Delta S°(T)$, $\Delta W°(T)$, and $\Delta G°(T)$ give rise to the classically observed behavior patterns in biological systems. $\Delta C_p°(+) \rightarrow \Delta C_p°(-)$ at (T_{Cp}), at low temperature. The implication is that the negative Gibbs free energy minimum at a well-defined (T_s), where the bound unavailable energy $T\Delta S°(T) = 0$, has its origin in the hydrophobic interactions, which are highly dependent on the details of molecular structure. We have shown in our work the existence of a thermodynamic molecular switch in pairwise sequence-specific hydrophobic interactions. Indeed, all interacting biological systems that we have thus far examined using the Planck-Benzinger approach point to the universality of this thermodynamic molecular switch [Chun, P.W.(2005) Physica Scripta T119, 219-225].

4P-B-084 MD validation of a model for short α-neurotoxin bound to nicotinic acetylcholine receptor from *Torpedo californica*

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Although the spatial structures of short-chain α-neurotoxins from snakes are known the accurate structure of the complex is not yet known. Here we present a molecular dynamics (MD) validation for the model of a short α-neurotoxin, neurotoxin II from *Naja oxiana* (NTII), bound to *Torpedo californica* muscletype nicotinic acetylcholine receptors (nAChR). It was built by comparative modeling and docking as described in [1]. The refinement of the constructed model was done on the basis of computer simulations. The runs were done for the system comprising α- and γ-subunits of nAChR and the toxin disposed according to the docking simulations.

During the MD calculation, all elements of secondary structure were well preserved and most of the contacts between the NTII and nAChR residues found by docking simulation were generally retained during MD operations. The toxin molecule squeezed a bit further between the subunits. The highly conserved and structurally stable cysteine-rich core of the toxin, which was initially more distant from the receptor's subunits, approached the γ-subunit, resulting in a decrease by 10° of the angle between the principal molecular axes of the toxin and receptor. MD simulation also confirmed five contacts of particular interest between residues of the receptor and NTII which seem to determine mainly their specific interaction. Further models will be applied for the rational design of new antagonists of nAChR and will be also tested by MD as the final verification tool.

References:

[1]. Mordvintsev D.Yu. et al. Comput. Biol. Chem., 2005, 29 (6), 398-411.