The molecular binding approach suggests that tripeptides are preferably located near hydrophobic residues of Aβ fibrils. The DC50 values determined from dose-response curves were found in micromolar range, the lowest values were found for tripeptides containing Proline. Experimental results confirmed theoretically predicted significance of Proline in tripeptide sequence.

Acknowledgement: This work was supported by grants VEGA 0181, No 2011/01/B/NZ1/01622, ESF 26110230061 and 26110230097, APVV 0171-10 and VVGS 98/13-14.

305-Pos Board B60

Efflux Time Courses of Cytosolic Proteins from Rabbit Skinned Muscle Fibers Reflect Dissociation of Enzyme Complexes

David Maughan[1], Brian Carlson[2], Jim Vigoreaux[3].
[1]Molecular Physiology/Biophysics, University of Vermont, Burlington, VT, USA, [2]Biotechnology and Bioengineering Center, Milwaukee WI USA, Medical College of Wisconsin, Milwaukee, WI, USA, [3]Biology, University of Vermont, Burlington, VT, USA.

In many multi-system metabolic diseases characterized by mitochondrial dysfunction (e.g. Myalgic Encephalomyelitis), glycolysis appears to be an important compensatory pathway for generating ATP. It is postulated that glycolytic enzymes form a complex that enhances the rate of ATP production through substrate channeling of metabolic product intermediates. To search for evidence of a reduced diffusion coefficient indicative of supramolecular complexes, we examined the efflux of endogenous glycolytic enzymes from rabbit psoas muscle fibers. Single fiber segments were skinned in oil and transferred to physiological salt solution. Cytosolic proteins that diffused into the solution were separated by gel electrophoresis and compared to load-matched standards for quantitative analysis. A radial diffusion model incorporating the dissociation and dissipation of supramolecular complexes accounts for an initial lag and subsequent efflux of glycolytic enzymes. The model includes terms representing protein crowding, myofilament lattice hindrance, and cytomatrix binding. Optimization of model to data returned estimates of apparent diffusion coefficients that were very low at the onset of diffusion ($\sim 10^{-10}$ cm^2 s^{-1}) but increased with time as cytosolic protein density decreased. The initial values are consistent with the presence of complexes *in situ*; higher later values (e.g., 0.2×10^{-7} cm^2 s^{-1} for phosphofructose kinase), with molecular sieving and transient binding of dissociated proteins. Channeling of metabolic intermediates via enzyme complexes may enhance production of ATP at rates beyond that possible with randomly distributed enzymes, thereby matching supply with demand. Metabolic channeling may allow glycolysis to better compensate for reduced ATP production in aerobic metabolic diseases.

306-Pos Board B61

Phase Diagram to Illustrate Protein Aggregation Profile and Conditions

Bin Li[1], Siqi Li[2], Yang Cao[2], Shaohua Xu[2].
[1]Florida Institute of Technology, Melbourne, FL, USA, [2]Department of Biological Sciences, Florida Institute of Technology, Melbourne, FL, USA.

Protein self-assembly and formation of amyloid fibers and/or amorphous aggregates is an early event in numerous human diseases, such as Alzheimer's disease, Parkinson's disease, and cataracts. Identification of the structural features generated in the aggregation process, especially under conditions similar to the tissue's viscous and crowded environment, helps to elucidate the mechanism of protein aggregation and the pathogenesis of these diseases. By systematically testing a broad range of conditions and construction of a 3-dimensional phase diagram, we identified the pH, salt, lysozyme concentration, and incubation time, for lysozyme to form amyloid fibers, amorphous aggregates, and gels. We examined the effect of viscosity and molecular crowding on lysozymes' aggregation profile. We characterized the aggregates by use of AFM, TEM, FPLC, and Thioflavin T binding assays, and found that amyloid fibers are formed between pH 2.0 and 3.0, amorphous aggregates at pH 3.5 and above. Glycerol or polyethylene glycol inhibits fiber formation. Gels are formed when fiber concentration is high, and the presence of glycerol or polyethylene glycol lowers the minimum fiber concentration required for gelation. Salt or shaking promotes amyloid fiber formation and shortens the time needed for gelation. Colloidal spheres are present in amyloid fiber solutions, as predicted by the linear colloidal aggregation model we introduced previously. The phase diagrams provide a comprehensive and clear picture of the relation between various aggregates and the conditions for their formation.

307-Pos Board B62

In Vitro Interactions Between Amyloid Beta and Islet Amyloid Polypeptide

Leah Vandiver.
Biomedical Engineering, Washington University in St. Louis, St. Louis, MO, USA.

Alzheimer's disease and type II diabetes mellitus are two prevalent protein misfolding diseases. Their co-morbidity has raised questions regarding potential interactions between the peptides that are implicated in each disease state. Islet amyloid polypeptide (IAPP) aggregates in type II diabetes mellitus while amyloid beta 1-42 (Aβ42) aggregates in Alzheimer's disease. Interactions between these amyloidogenic peptides may result in coaggregation that could exacerbate the cellular toxicity associated with each disease by increasing the amount of toxic oligomers over less toxic mature fibrils.

We tested this hypothesis *in vitro* by studying aggregation kinetics, aggregate morphology, stability and cytotoxicity of IAPP / Aβ42 mixtures. Tetramethylrhodamine (TMR)-labeled Aβ42 was incubated with excess AlexaFluor 488 (A488)-labeled IAPP which revealed through colocalization analysis that both peptides coaggregate. A complimentary experiment with excess TMR-labeled Aβ42 showed similar results. We then monitored aggregation kinetics of IAPP and Aβ42 through Thioflavin T fluorescence. Equimolar mixtures of Aβ42 and IAPP displayed aggregation kinetics intermediate of Aβ42 and IAPP. While IAPP prolonged the lag-phase of Aβ42, the opposite was not observed. At the same time, the presence of equimolar or excess IAPP diminished the SDS-resistance of fibrillar Aβ42 aggregates. We then added aggregation intermediates corresponding to the lag phase, early and late growth phases, and the plateau phase to human neuroblastoma (SH-EP) cells to test for cytotoxicity. Surprisingly, the cytotoxicity of Aβ42 / IAPP mixtures was intermediate to that of Aβ and IAPP alone. Taken together, these results indicate a molecular interaction exists between these two disease-relevant peptides and may provide a link between type II diabetes mellitus and Alzheimer's disease but does not support a model in which co-aggregation with IAPP directly exacerbates Aβ toxicity by increasing oligomer formation.

308-Pos Board B63

NADH is an Endogenous Reporter for Alpha-Synuclein Aggregation in Live Cells

Nicoletta Plotegher[1], Chiara Stringari[2], Sohail Jahid[3], Enrico Gratton[3], Luigi Bubacco[1].
[1]Department of Biology, University of Padova, Padova, Italy, [2]Laboratory for Optics and Biosciences, École Polytechnique, Paris, France, [3]Laboratory for Fluorescence Dynamics, University of California, Irvine, CA, USA.

Alpha-synuclein aggregation is amply investigated for its involvement in Parkinson's disease etiopathogenesis. It has been shown that alpha-synuclein monomers, under pathological conditions, self-assembly to form oligomeric species that further aggregate into amyloid fibrils. Alpha-synuclein fibrils are the main constituent of Lewy Bodies, which are one of the characteristic hallmarks of Parkinson's disease.

Alpha-synuclein aggregation is studied in vitro and in cellular models with the aim to correlate toxicity mechanisms to defined aggregation products. However, the characterization of the aggregation process in cells is a difficult task that typically needs cell lysis or fixation, or the use of exogenous dyes.

Moreover, several different toxic mechanisms were ascribed to alpha-synuclein aggregates, i.e. clearance mechanisms impairment, mitochondrial dysfunctions, oxidative stress, neuroinflammation. In particular, mitochondria seem to be a target for alpha-synuclein to exert its toxicity. Several independent results suggested that alpha-synuclein overexpression and/or aggregation may cause impairment of cellular metabolism due to mitochondrial fragmentation and complex I dysfunction.

On these premises, we report here the results obtained from the characterization of NADH fluorescence properties variation in vitro and in cell models during alpha-synuclein aggregation.

The application of the phasor approach for the study of NADH fluorescence lifetime and spectra allowed the determination of specific variation in the NADH fluorescence properties correlated to alpha-synuclein oligomerization and amyloid fibrils formation in vitro and in live cells.

The results presented here suggest that alpha-synuclein aggregation may be associated to impairment in cell metabolism due to damage to complex I in mitochondria and disruption of NADH and NAD^+ equilibrium. Moreover, NADH can be used as an endogenous fluorescence reporter for alpha-synuclein aggregation in vitro and in cellular models.

(Work supported in part by NIH grants P50 GM076516 and P41 GM103540, and by Aldo Gini Foundation.)