gating in human $Ca_V1.2$ channels, we have optically tracked the activation of the S4 helices of repeats I, III and IV by site-directed fluorescent labeling of introduced Cysteines with thiol-reactive probes. The channels were co-expressed with their modulatory subunits β_3 and $\alpha_2\delta$ in *Xenopus* oocytes. Ionic current and fluorescence emission were simultaneously recorded using the cut-open oocyte voltage clamp fluorometry technique. Prior voltage-clamp, oocytes were injected with 100 nl 80 mM BAPTA.4K, to prevent the activation of endogenous Ca^{2+}-gated Cl^- channels. The extracellular solution contained 2 mM Ba^{2+} or 10 mM Ca^{2+} as charge carrier. Gating currents were recorded by replacing Ca^{2+} or Ba^{2+} with Co^{2+}. 0.1 mM ouabain was added to abolish Na^+/K^+ ATPase non-linear charge movement. Voltage-dependent fluorescence changes (ΔF) were reported by fluorophores attached to substituted Cysteines at the extracelular end of the S4 segment in repeats I, III and IV, tracking local, voltage-dependent conformational rearrangements. The voltage dependence of observed fluorescence deflections preceded ionic activation, reporting repeat-specific VSD transitions taking place during shut states of the channel. Prolonged sojourns at depolarized potentials (+50 mV) shifted the voltage-dependence of reported ΔF to more hyperpolarized potentials by >50mV, recapitulating previously-characterized gating current properties. In summary, we report the first optical characterization of VSD conformational changes in a human voltage-gated Ca^{2+} channel, revealing repeat-specific voltage- and time-dependent properties.

1840-Plat

Engineering the Composition of L-Type (Cav1.2) Channels in Heart Cells using Split Intein-Mediated Protein Trans-Splicing

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Ca^{2+} influx through L-type ($Ca_V1.2$) channels in heart regulates excitation-contraction (EC) coupling, action potential duration, and gene expression. This versatility of L-type channels in heart is hypothesized to be mediated in part by differential sub-cellular compartmentalization and hormonal modulation of distinct $Ca_V1.2$ channel pools in cardiac myocytes. Such prevailing hypotheses are best tested directly in cardiac myocytes, since their unique cyto-architecture and signaling environment cannot be replicated in heterologous cells. There are two major hurdles: first, the large size of pore-forming α_{1C} subunits exceeds the packaging capacity of viral vectors, necessary to express exogenous $Ca_V1.2$ channels in adult cardiac myocytes; second, endogenous channels are a source of confounding contaminating signals. To overcome these limitations, we focused on functionally reconstituting two separately expressed moieties of the channel using a novel split-intein-mediated protein splicing approach. In HEK 293 cells, split-intein fragments were *trans* spliced to generate full-length α_{1C} as determined by Western blot. When co-expressed with auxiliary β subunits, *trans*-spliced α_{1C} trafficked normally to the cell surface and yielded robust whole-cell currents (I_{Ca}). The split-intein fragments were readily incorporated into adenoviral vectors which when used to infect adult myocytes yielded *trans*-spliced α_{1C} that was detected at the cell surface. To isolate exogenous I_{Ca}, we introduced mutations that reduced dihydropyridine sensitivity of *trans*-spliced α_{1C} (α_{1CDHP-}). In the presence of 10 μM nifedipine, myocytes expressing *trans*-spliced α_{1CDHP-} yielded significantly larger currents than controls expressing *trans*-spliced α_{1C}. The results demonstrate a novel approach to robustly engineer the pore-forming α_{1C} subunit composition of $Ca_V1.2$ channels in cardiac myocytes, removing a longstanding technical obstacle to L-type channel structure-function studies in heart.

Platform: Protein Assemblies & Aggregates

1841-Plat

Toxic Intermediates in Islet Amyloid Formation: Analysis of IAPP Mutants Reveals a Correlation between Lag Time and Toxicity

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Islet amyloid polypeptide (IAPP, Amylin) is responsible for amyloid formation in type 2 diabetes and in islet cell transplants. The mechanism of IAPP toxicity is poorly understood. Here we identify and characterize the toxic species produced during amyloid formation by human IAPP. using a combination of time-dependent biophysical and biological assays, we show that the transiently populated pre-fibrillar intermediates formed in the early phase of amyloid formation are toxic and they are loosely packed with very modest amounts of secondary structure. The only known natural mutation found in mature human IAPP is a Ser20-to-Gly mis-sense mutation, which appears to be associated with an increased risk of early-onset type 2 diabetes. We demonstrate that the mutant accelerates amyloid formation under physiologically relevant conditions. We rationally design another variant at residue 20, S20K-IAPP, which is much slower to aggregate with an 18-fold longer lag phase and inhibits wild type amyloid formation. The pronounced effects of the Ser20 mutants highlight the sensitivity of amyloid formation to the identity of position 20. By comparing the kinetics and toxicity profiles of the two Ser20 mutants to wild type IAPP, we demonstrate that changes in the length of the lag phase directly correlate with changes in the onset and the duration of toxicity. Our findings provide direct evidence that the toxic species are transient intermediates and have implications for the treatment of type 2 diabetes and other amyloid related diseases.

1842-Plat

Solid-State NMR Study of Pathologically Relevant Amylioid Intermediate of 42-Residue Alzheimer'S Beta

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Accumulating evidences suggest that many neurodegenerative diseases including Alzheimer's disease (AD) are linked with cytotoxic diffusible aggregates of amyloid proteins, which are metastable intermediate species in protein misfolding. Despite increasing importance of the amyloid intermediates, very little has been known on their structures, relationship with amyloid fibril, and pathogenic functions. This work presents a site-specific structural study on an intermediate called amylo-spheroid (ASPD)(1) for 42-residue Alzheimer's β (Aβ(1-42)) by solid-state NMR (SSNMR), which offers means to characterize metastable amyloid intermediates.(2) As the ASPD level in a brain correlates with the severity of AD, ASPD is likely an intermediate pathogenically relevant to AD.(1) We demonstrate that detailed structural examination by ^{13}C SSNMR is possible on synthetic ASPD that well mimics native ASPD isolated from a brain extract from an AD patient. Electron micrograph and immunological analyses using an ASPD-specific ''conformational'' antibody confirm that morphologies and conformations of the synthetic ASPD used for the present NMR analysis are similar to native ASPD. ^{13}C SSNMR chemical-shift analysis over 20 residues demonstrated that ASPD is made of a homogeneous conformer that is largely composed of β-sheet structure. An inter-strand ^{13}CO-^{13}CO distance measurement suggests that the ASPD involves a parallel β-sheet arrangement despite the fact that ASPD does not bind to fibril-specific dyes such as thioflavin-T. The structural features of ASPD will be compared with those of amyloid fibril for Aβ(1-42), which were elucidated by SSNMR analysis. The approach presented here is likely to open an avenue to examine structural details of various amyloid intermediate species pathologically relevant to AD or other amyloid diseases, for which structures have been poorly characterized.

References
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1843-Plat

Single Molecule Fluorescence Studies of Amyloid Beta 1-42 Aggregation

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The proteolytic cleavage of the transmembrane amyloid precursor protein (APP) produces amyloid-β peptides (Aβ) that vary from 38 to 43 amino acids in length. Two of these peptides, $A\beta_{1-40}$ and $A\beta_{1-42}$, are the major components of the extracellular amyloid plaques characteristic of Alzheimer's disease (AD). Within these plaques, the Aβ is found aggregated into long polymeric assemblies rich in β-sheet structure that are known as amyloid fibrils. Although the correlation between plaque load and disease severity is poor there is strong evidence that small soluble oligomers of Aβ formed during the early stages of the aggregation process are the agents of AD-associated neurotoxicity (1). Single molecule fluorescence techniques have the potential to resolve the size and structural heterogeneity of these oligomers, which are often difficult to discern by ensemble methods. Most importantly, they allow the characterisation of small oligomeric species at the nucleation stage of the aggregation as the structures of amyloid seeds remain ambiguous (2). Equimolar mixtures of $A\beta_{1-42}$ singly labelled with either HiLyteFluor-488 or HiLyteFluor-647 were studied using