cell membrane binding sites enables prothrombinase formation with consequent meizothrombin formation; meizothrombin then functions as an amplifier of the process of factor V activation and thus has an important procoagulant role.

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78.28

An Analysis of pH Effects on the Hepatitis C Virus NS3 Protein' Reveals a Molecular Basis for Helicase Movement

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Hepatitis C Virus NS3 protein is a serine protease and a helicase capable of unwinding RNA and DNA. To better understand the mechanism of the NS3 helicase function, its distinctive pH profile was examined. Experiments were performed with both the full-length NS3 protein with its NS4A cofactor and a truncated protein lacking the protease domains. Both proteins unwind RNA best at pH 6.5 but little to no unwinding can be detected at neutral or physiological pH. HCV helicase catalyzed DNA unwinding is less pH sensitive than RNA unwinding, but is also fastest at ph 6.5. Most thermodynamic and kinetic parameters describing helicase action are not influenced by pH. However, the binding of HCV helicase to RNA (and DNA) in the presence of ATP, which decreases the affinity of HCV helicase for DNA, is dramatically weaker at a higher pH, suggesting that an acidic residue is involved in pushing the helicase along the nucleic acid backbone. Structure-based mutagenesis was used identify such a residue. When glutamine-493 is changed to a lysine (E493K), the protein binds DNA more tightly in the presence of ATP and unwinds a double helix poorly compared with wildtype. E493K catalyzed unwinding is, however, not as pH sensitive and at physiological pH, E439K retains some ability to unwind both RNA and DNA. The results support a "propulsion-by-charge-repulsion" mechanism whereby movements of the helix containing Glu493 upon ATP binding propel the helicase along nucleic acid.

78.29

Conformational flexibility and distance effects determine kinetic distinctions among aldolase isozymes

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Vertebrate aldolase exists as three isozymes (A, B, and C) with distinct kinetic properties consistent with their physiological role and tissue-specific expression. The structures of the isozymes reveal that their active sites are nearly identical, suggesting that amino acids involved in the kinetic differences lie outside of this area. A sequence alignment revealed residues that are specific to each isozyme, or isozyme-specific and glycolaldehyde, of which HP is the precursor for the biosynthesis of folate cofactors. Like other enzymes in the folate biosynthetic pathway, DHNA is an attractive target for developing antimicrobial agents. As an aldolase, DHNA is also unique in that it requires neither the formation of a Schiff base between the enzyme and its substrate nor metal ions for catalysis. Furthermore, the enzyme also catalyzes the epimerization of the substrate DHNP to generate 7,8-dihydromonapterin at a substantial rate. While the crystal structures of the apo DHNA from *Staphylococcus aureus* and its product complex have been reported, the critical interactions involved in DHNA catalysis have not been revealed. To elucidate these interaction, molecular dynamics simulations with explicit solvent molecules have been performed on the apo DHNA, its substrate complex, and the product complex. The simulations have revealed conformational changes in the catalytic cycle and identified general acid and bases in the catalysis. Supported by NIH grant GM58221 (H.Y.).

78.31

C-reactive Protein Rise is Associated with the Development of Acute Events in a Model of Plaque Rupture and Thrombosis

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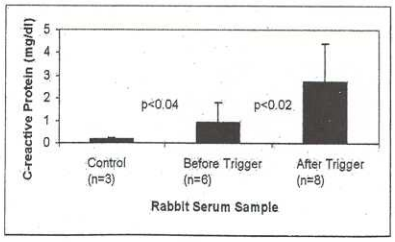
Elevated levels of C-reactive protein (CRP) have been associated with increased risk for development of cardiovascular events. In order to follow the trend of CRP over the course leading to an acute event, we evaluated CRP levels under three conditions: normal rabbits, atherosclerotic rabbits before and after pharmacological triggering of plaque rupture and thrombosis. As previously reported, plaque rupture and thrombosis is induced using Russell viper venom (RVV) and histamine in an atherosclerotic rabbit model.

Methods: Atherosclerosis was induced with balloon deendothelialization and feeding a high cholesterol diet for 9 months. Serum samples were obtained from control rabbits (n=3), and atherosclerotic rabbits, before (n=6) and 48 hours after RVV and histamine-induced thrombosis (n=8). Rabbit specific high sensitivity ELISA was developed to detect the levels of serum CRP concentrations.

Results: CRP levels were significantly lower in control normal rabbits compared to rabbits with atherosclerotic plaques. Our results further demonstrate that rabbits with RVV and histamine-triggered thrombosis had



significantly higher levels of serum CRP than non-triggered atherosclerotic rabbits.

Conclusion: The rise of serum CRP levels both after cholesterol feeding and the sudden rise after pharmacological triggering of thrombus may help using of CRP to evaluate not only the long-term risk but also a more short-term risk of events if CRP levels increase acutely.

78.32

Targeted prodrug chemotherapeutics bypass p-glycoprotein resistance and kill tumors in vivo with high efficacy and target-dependent selectivity

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Doxorubicin intercalates into DNA, causes double strand breaks, and leads to apoptotic death. Limitations to the efficacy and therapeutic index of Doxorubicin include poor tumor selectivity, high systemic toxicity, and the development of resistance, especially p-glycoprotein-mediated. We chemically coupled Doxorubicin to n antibody directed to the Insulin-Like Growth Factor-1 receptor. We also coupled Taxol to a small molecule synthetic ligand of HER-2 receptors. Both IGF-1R and HER2 are receptors expressed in many tumors and ar validated as a tumor targets.

The prodrug conjugates bound to tumor cells selectively, and accumulated efficiently and only in receptor-expressing cells. The conjugate was processed to release free Doxorubicin inside target cells leading to selective toxicity, had >200-fold improved therapeutic index, and in vivo reduced tumor load with no systemic toxicity. Importantly,

78.34

Novel function for receptor activity modifying proteins (RAMPs) in post-endocytic receptor trafficking

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RAMPs (1-3) are single transmembrane proteins crucial for plasma membrane expression and receptor phenotype of various GPCRs. For example, adrenomedullin receptors are comprised of RAMP2 or 3 (AM1R and AM2R, respectively) and calcitonin receptor-like receptor (CRLR), while a CRLR heterodimer with RAMP1 yields a CGRP receptor. The major aim of this study was to evaluate the role of RAMPs in receptor trafficking. We hypothesized that a PDZ domain present in the C-terminus of RAMP3, but not RAMP1/2, leads to protein-protein interactions that determine receptor trafficking. N-ethylmaleimide sensitive factor (NSF) has been shown to regulate the recycling of several receptors via protein-protein interactions. Employing adenylate cyclase assays, radioligand binding, and immunofluorescence microscopy in HEK293 cells, we have observed that the CRLR-RAMP complex undergoes agonist-stimulated desensitization and internalization, followed by degradation of the receptor complex. Co-expression of NSF with the CRLR-RAMP3 complex, but not CRLR-RAMP1/2, altered receptor trafficking to a recycling pathway. Mutational analysis of RAMP3 indicated that the PDZ domain of RAMP3 interacts with NSF to cause the change in trafficking. Furthermore, substitution of the RAMP3 PDZ domain on the C-terminus of RAMP2 converted the receptor complex to a recycling phenotype with NSF co-expression. Our findings provide the first functional difference between the AM1R and AM2R. These results