Factor VII and other Vitamin K-Dependent Proteins with Enhanced Function

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Outline:

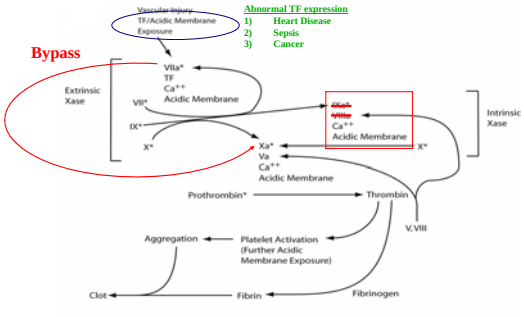
1) Mechanism of membrane contact

2) Enhancement of membrane binding by mutagenesis

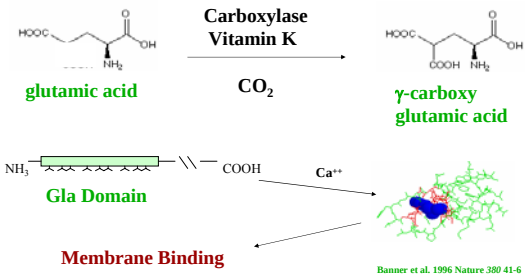
3) Assay of enhanced proteins

4) The nature of the biological membrane

Factor VIIa and TF Initiate the Blood Coagulation Cascade



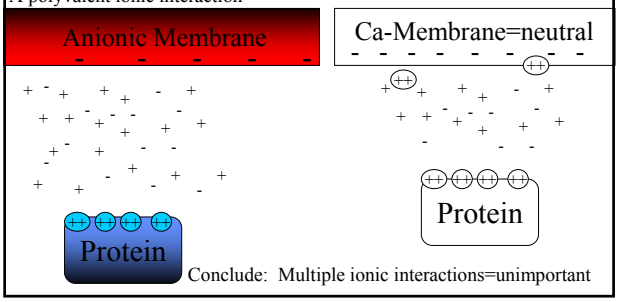
Vitamin K-Dependent Proteins Contain γ-carboxy glutamic acid (Gla) which Facilitates Binding to Acidic Membranes



Binding is not influenced by NaCl as long as Ca is saturating (~1980)

Ionic strength should interfere with A polyvalent ionic interaction

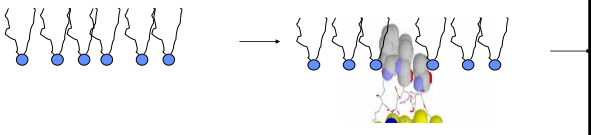
No impact of NaCl on binding When both were Ca saturated



Conclude: Multiple ionic interactions=unimportant

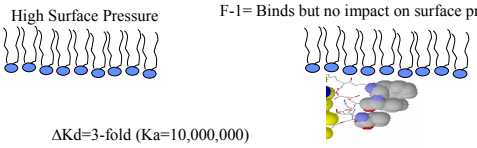
Fragment 1 Binding to Phospholipid Monolayers (~1982)

Protein Insertion causes surface pressure change



High Surface Pressure

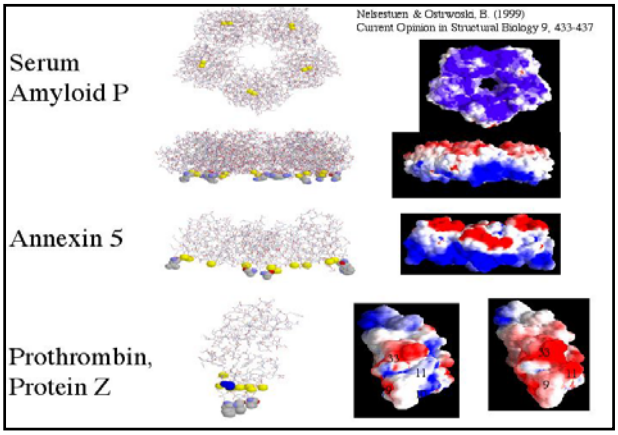
F-1= Binds but no impact on surface pressure



ΔKd=3-fold (Ka=10,000,000)

ΔΔG= +0.65 kcal/mol
(ΔG= -11.0 kcal/mol)

Conclude: Insertion is not important



Sequence Homology and Membrane Binding Properties of Vitamin K-dependent Proteins

1		10	20	30	40	Protein
ANS	_LL γγI	KQG NLγ	RγC Iγγ	LCN Kγγ	ARγ VFγ NDP γTD YFY PKY	hS
AGS	YLL γγL	FγG HLγ	KγC Wγγ	ICV Yγγ	ARγ VFγ DDγ TTD γFW RTY	bZ
ANS	_FL γγV	KQG NLγ	RγC Lγγ	ACS Lγγ	ARγ VFγ DAγ QTD γFW SKY	bX
ANK	GFL γγV	RKG HLγ	RγC Lγγ	PCS Rγγ	AEγ ALγ SLS ATD AFW AKY	bPT
AGS	YLL γγL	FγG NLγ	KγC Yγγ	ICV Yγγ	ARγ VFγ NγV TTD γFW RRY	hZ
YNS	GKL γγF	VQG NLγ	RγC Mγγ	KCS Fγγ	ARγ VFγ NTγ KTT γFW KQY	bIX
ANS	_FL γγL	RHS SLγ	RγC Iγγ	ICD Fγγ	AKγ IFQ NVD DTL AFW SKI	hC
ANA	_FL γγL	RPG SLγ	RγC Kγγ	QCS Fγγ	ARγ IFK DAγ RTK LFW ISY	hVII

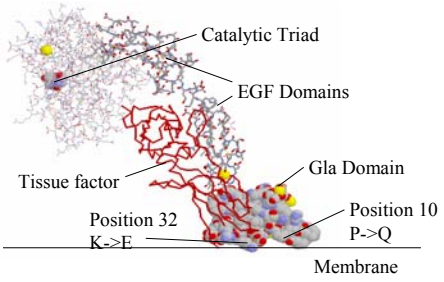
γ = γ-carboxy glutamic acid (Gla)

Gla Domains are Highly Homologous

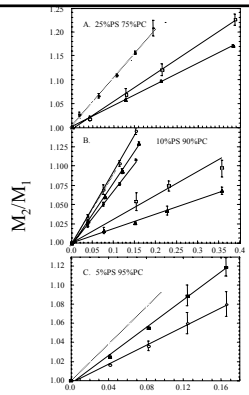
Membrane Affinity

Protein	K_d (nM)
hS	51
bZ	32
bX	40
bPT	110
hZ	170
bIX	1000
hC	1500
hVII	1700

Part I. The Membrane binding site



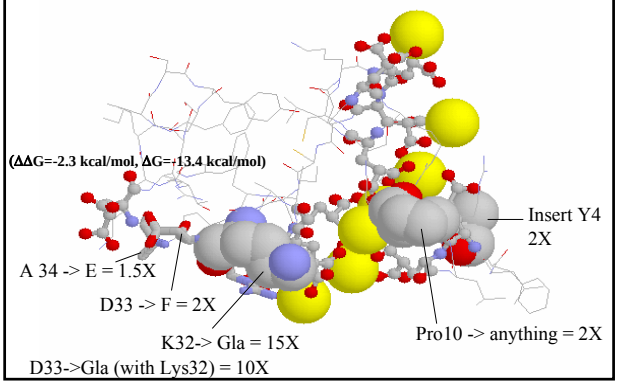
Banner et al., 1997



Membrane binding By factor VII Mutants:

Affinities have to be Assessed in Stages Due to Extreme Differences

Changes that enhance membrane affinity of FVII(a)

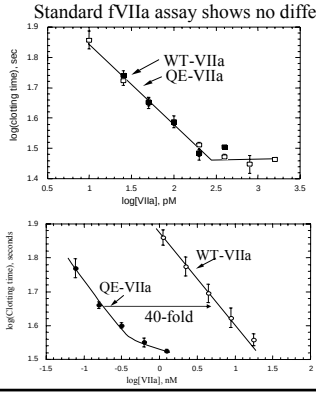


Conclusions

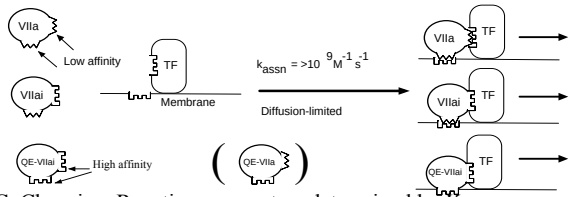
- Site directed mutagenesis of the Gla domain can improve function by 200-fold (or more?)
- The meaning of this to the membrane binding mechanism is unclear

Part II. Assay Methods

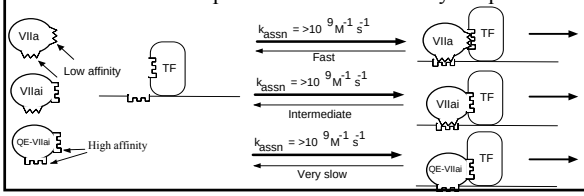
Standard fVIIa assay shows no difference



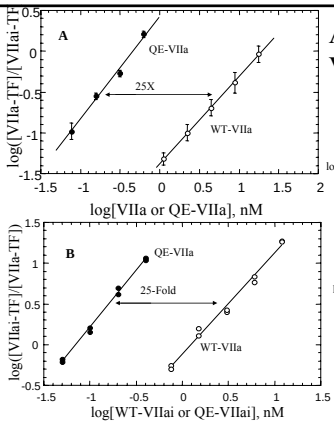
A. Acute: Reaction parameters determined by kassn.



C. Chronic: Reaction parameters determined by Keq.



Analysis of VIIa or VIIai by Xa production



VIIa/TF (in the presence of VIIai)

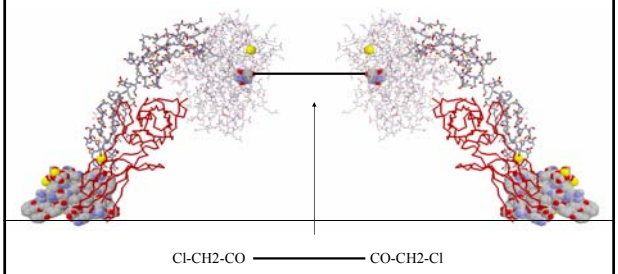
X ⟶ Xa

log(VIIa-TF/VIIai-TF)=logKd1/Kd2
+ log(VIIa)-log(VIIai)

log(VIIai-TF/VIIa-TF)=log(Kd2/Kd1)
+log(VIIai)-log(VIIa)

(VIIa) and (VIIai)=free concentrations

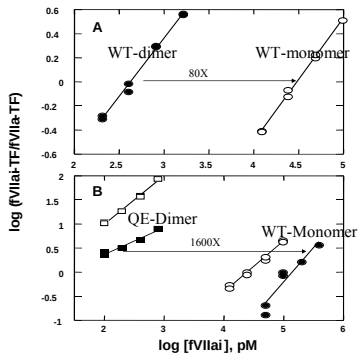
Dimerization of FVIIai through the active sites



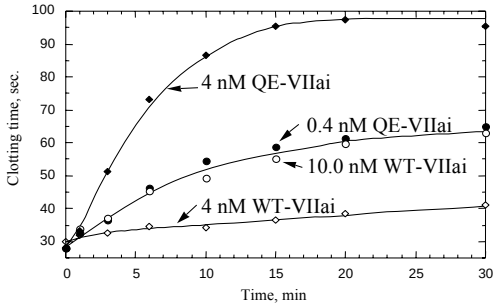
Cl-CH2-CO ——— CO-CH2-Cl

Bifunctional, active site-directed reagent

Activity of Dimeric fVIIai PEG Crosslinked



FVIIai is a Delayed-action inhibitor



Conclusions Part II

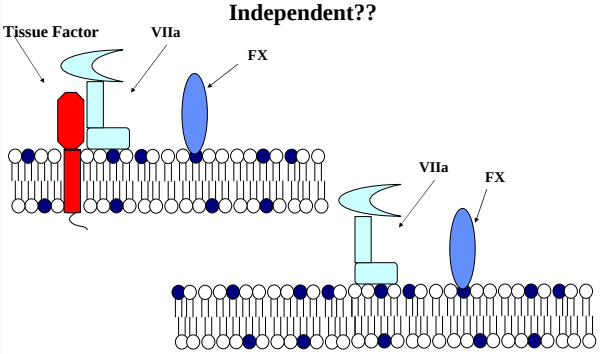
- TF expressed on Innovin assembles with fVIIa at the collisional limit
- Enhanced functions of fVIIa(i) mutants are only detected under equilibrium conditions
- This could be a major benefit for fVIIa(i) with potential therapeutic benefits
- Or, does Innovin produce *in vitro* phenomena??

Part III.
What is the mechanism of action of fVIIa
What is the nature of the biological membrane?

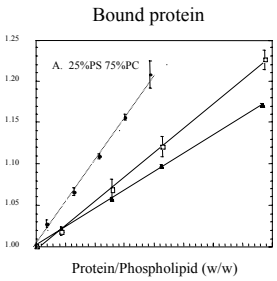
If it has high affinity for vitamin K-dependent proteins:
•It will produce a high density of protein on the membrane
•WT-VIIa will operate at nearly maximum rate
•Mutants will have very little advantage

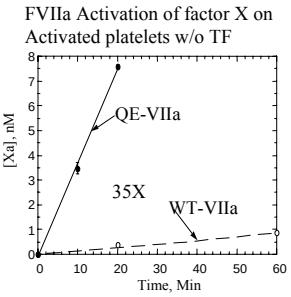
If is has low affinity
•Protein density on the membrane will be low
•Mutants will show maximum enhancement in function

Factor VIIa therapy: Tissue factor-dependent or Independent??

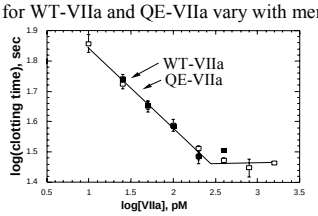


The activated platelet provides a low affinity surface for fVIIa

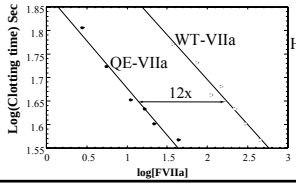




Results for WT-VIIa and QE-VIIa vary with membrane-TF used.



Innovin as the Membrane

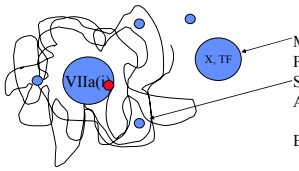


Human Brain Thromboplastin

Conclusions part III

- The biological membrane appears to be a low affinity surface for Vitamin K-dependent proteins
- This will maximize the benefit of membrane site mutants that have high membrane affinity.
- The membrane compositions of most *in vitro* assay kits are optimized for the *in vitro* assay and may not mimic the properties of biological membranes

Part IV. Polyethylene Glycol derivatives of factor VIIa(i)



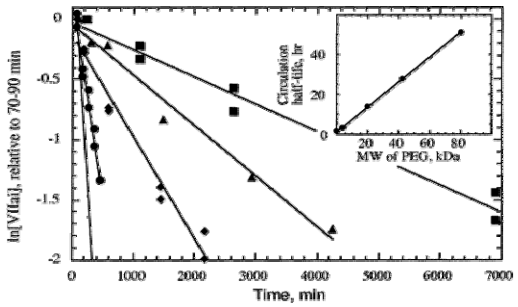
Macromolecules have a difficult time
Penetrating the crowded environment
Small molecules can reach the
Active site very easily

Benefits: Lowered antigenicity
Longer circulation lifetime

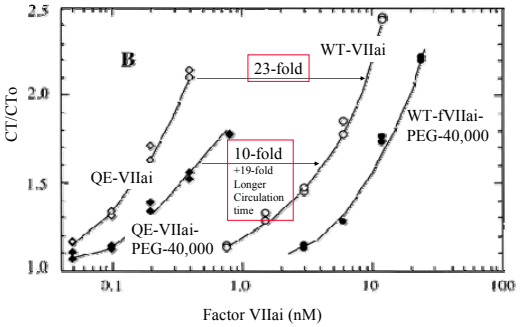
PEG-modified protein

What are the tradeoffs for PEG modification of fVIIa(i) molecules?

Circulatory lifetime of factor VIIai: active site modified with PEG



Benefit of PEG-modified fVIIai



Conclusions:

- Membrane binding affinity of FVII can be increased by 200-fold.
- Function at equilibrium is closely related to membrane affinity.
- Factor VIIa-TF association occurs at a maximum rate.
- Most improvements (45-fold) are close to Position 32.
  - Deletions and insertions around position 32 are devastating.
  - Similar changes in the omega loop retain activity.
- Dimeric VIIai has 90-fold higher function than monomeric VIIai.
- It does not yet appear possible to reconcile structures with detailed biochemical properties.
  - Number of calcium ions in the complex.
  - Proline isomerization in prothrombin fragment 1.
  - Mutagenesis does not provide an unambiguous pattern.
- There is an extended future in studying the mechanism of membrane association by vitamin K-dependent proteins.

Acknowledgements:

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