Interaction of human fibrinogen receptor (GPIIb-IIIa) with decorsin[1]

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KEY WORDS platelet glycoprotein GPIIb-IIIa complex; decorsin; drug design

ABSTRACT

AIM: To build up the structure of human fibrinogen receptor GPIIb-IIIa, subsequently combined with its antagonist decorsin, and to investigate the interaction between decorsin and its receptor GPIIb-IIIa at the molecular level. **METHODS:** A three-dimensional (3D) molecular model of human fibrinogen receptor GPIIb-IIIa was generated by InsightII, a distance geometry-based homologous modeling package. The structure of human fibrinogen receptor GPIIb-IIIa was built by the InsightII/Homology module using the corresponding of integrin *alpha*V*beta*3 (PDB filecode 1JV2) as the template. Then the primary structures were optimized by energy minimization. Subsequently the structural model was docked with its antagonist decorsin (PDB filecode 1dec). **RESULTS:** A good substrate-receptor interaction model was achieved. The interaction sites with decorsin converge at domain 8 (βA domain of β3 subunit) of GPIIb-IIIa. The direct interatomic contacts were made between 16 GPIIb/IIIa residues and 10 decorsin amino-acid residues. These included van der Waals contacts, electrostatic interaction, hydrogen bond, and salt bridge. Residues in contact were concentrated in four dispersed regions of human GPIIb-IIIa: the RGD reaction motif (118-132 of GPIIIa), the span from 210 to 213 of GPIIIa, Thr182 residue and Asp251 residue of GPIIIa; and they were distributed over five segments of decorsin: Asp10 residue, Asn18 and Lys19 residues, Arg28 residue, RGD motif, and Asp35-Pro36-Tyr37 segment. **CONCLUSION:** This complex model plays an important role in development and research of some new drugs, especially a new guided fusion-type fibrinogen receptor antagonist.

INTRODUCTION

Current comprehension of the pathophysiological mechanism of atherosclerosis recognizes platelet aggregation as a major cause of thrombus formation in patients with myocardial infarction. Platelet aggregation essentially requires fibrinogen, which is a major adhesive macromolecule that links platelets through binding to GPIIb-IIIa after a constellation of stimuli, binding with platelet glycoprotein IIb-IIIa (GPIIb-IIIa, fibrinogen receptor, integrin αIIbβ3), a receptor placed on the platelet membrane. Fibrinogen is a 340 kDa glycoprotein primarily synthesized by hepatocytes and secreted as a hexamer composed of three pairs of polypeptide chains (Aα, Bβ, and γ), encoded by three different genes clustered on chromosome 4q28. Two peptide sequences are involved in the binding of fibrinogen to GPIIb-IIIa: the RGD sequence present in fibrinogen (also in fibronectin, von Willebrand facter, and vitronectin) and the KQAGDV sequence at the gamma chain of

[1] Project supported by the National Natural Science Foundation of China, No 30171094 and No 30271497.
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Received 2003-08-08 Accepted 20004-04-06

fibrinogen, found exclusively in fibrinogen and probably the major site for interaction with GPIIb-IIIa[1-3]. GPIIb-IIIa is a heterodimer consisting of α and β subunits, belonging to integrin family. Integrins not only bind adhesive ligands, but also act as signaling receptors. Both functions allow the integrin αIIbβ3 to mediate platelet aggregation[4]. Platelet agonists (including ADP, epinephrine, thrombin, collagen, arachidonic acid, and PAF) activate αIIbβ3 (inside-out) to allow the binding of soluble fibrinogen. Subsequent platelet aggregation leads to outside-in αIIbβ3 signaling, which results in calcium mobilization, tyrosine phosphorylation of numerous proteins including β3 itself, increased cytoskeletal reorganization and further activation of αIIbβ3[5,6]. Thus, outside-in signals enhance aggregation, although the mechanisms and functional consequences of specific signaling events remain unclear. This knowledge has led to the development of GPIIb-IIIa antagonists as a logical strategy for inhibiting platelet aggregation and preventing coronary thrombosis.

Law *et al*[7] identified the integrin cytoplasmic tyrosine motif as a key mediator of β-integrin signal and a potential target for new therapeutic agents. The β3 subunit of αIIbβ3 contains two cytoplasmic tyrosine residues and is phosphorylated upon platelet aggregation. The tyrosines form part of the integrin cytoplasmic tyrosine binding (ICY) motif, consisting of two tyrosines separated by 11-19 residues with the upstream tyrosine in the context of $NpxY_{747}$ and the downstream tyrosine in $NxxY_{759}$, both potential phosphotyrosine binding (PTB) recognition sites. Three signaling pathways, they are, thromboxane, secreted ADP, and cAMP pathways may be involved in the binding pathway of fibrinogen and its receptor, while protein kinase C (PKC) activation seems to be the final common step of the three pathways. The increase of PKC activity can lead to activation of GPIIb-IIIa resulting in exposure of fibrinogen receptors, which may serve to convert this integrin into a functional receptor for fibrinogen[5,8]. PKC plays a crucial role in the induction of fibrinogen receptors, while inhibition of PKC activity can decrease fibrinogen binding to its receptor. These pathways mentioned will finally affect fibrinogen's binding to its receptor. Thus, GPIIb-IIIa antagonists can block fibrinogen binding to its receptor GPIIb-IIIa and inhibit the final step of platelet aggregation.

There are four classes of GPIIb-IIIa antagonists, including monoclonal antibodies (7E3[9]), polypeptides containing an RGD or KGD sequence isolated from snake venoms or leeches (decorsin[10]), low molecular weight linear or cyclic peptides containing either an RGD sequence or the carboxyl terminal sequence of the γ-chain of fibrinogen (eptifabatide[11]), and peptidomimetics or non-peptide antagonists (tirofiban, sibrafiban, and lamifiban)[11]. Decorsin is a 39-residue RGD-protein crosslinked by three disulfide brides isolated from the leech Macrobdella decora belonging to the family of GPIIb-IIIa antagonists and acting as a potent inhibitor of platelet aggregation[10,12]. Here, we constructed a structural model for human fibrinogen receptor using integrin *alpha*V*beta*3 (PDB filecode 1JV2)[13] as the template and a complex model of human fibrinogen receptor with its antagonist decorsin by molecular modeling, focusing on their interaction with decorsin and design of new guided fibrinogen receptor antagonist.

MATERIALS AND METHODS

Molecular modeling of human fibrinogen receptor Molecular modeling of the three-dimensional (3D) structures of human fibrinogen receptor was performed on a Silicon Graphics Iris O2 (SGI Inc, Silicon, CA, USA) workstation using the Homology modules of the commercial software packages InsightII 2000 (MSI, St Louis, MI, USA).

The amino acid sequences of integrin *alpha*2b (cd41, NM-000419, NP-000410.1) and *alpha*5 (cd51, NM-002210, NP-002201.1) were from Genebank and SWISS-PROT, which consist of 1309 and 1408 residues. The results of sequence alignment by BLAST showed there was higher homologous property between integrin *alpha*2b and *alpha*5, similarity nearly 54 % (Tab 1). One high-resolution X-ray crystal structures of integrin *alpha*V*beta*3 (αVβ3, PDB filecode 1JV2)[13] was used as template structures to create integrin *alpha*2b*beta*3 model using Homology module, where the fit-RMS deviation of subunit *alpha*2b with template *alpha*V is 0.8437 Angstroms. The whole protein structural models were optimized by molecular dynamics and molecular mechanics. First, the geometry of the protein was optimized for 200 steps with the steepest descent minimizer and subsequently for 2000 steps with the conjugate gradient minimizer, using the cvff force field with Kollmann All-atom charges. A cutoff of 0.8 NM was used, while dielectric constant was set 5.0 and dependent on the distances. Second, the structure was simulative annealed by molecular dynamics using

Tab 1. Alignment of the integrin alpha 2 and integrin alpha 5 [1].

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Alpha2: 19   LLLGPCAAPPAWALNLDPVQLTFYAGPNGSQFGFSLDFHKDS-HGRVAIVVGAPR--TLG 75
             LLL     P   A NLD       Y+GP GS FGF++DF   S   R+ ++VGAP-  T
Alpha5: 18   LLLSGLLLPLCRAFNLDVDSPAEYSGPEGSYFGFAVDFFVPSASSRMFLLVGAPKANTTQ 77

Alpha2: 76   PSQEETGGVFLCPWRAEGGQCPSLLFDLRDETRNVGSQTLQTFKARQGLGASVVSWSDVI 135
             P   E G V  C W +   -C  + FD            L+ FK+ Q  GASV S  D I
Alpha5: 78   PGIVEGGQVLKCDWSSTR RCQPIEFDATGNRDYAKDDPLE FKSHQWFGASVRSKQDKI 135

Alpha2: 136  VACAPWQHWNVLEKTEEAEKTPVGSCFLAQPESGRRAEYSPCRGNTLSRIYVENDFSWDK 195
             +ACAP  HW    K E   - PVG+CFL   +  +  EY+PCR   +          +  +
Alpha5: 136  LACAPLYHWRTEMKQE---REPVGTCFLQ--DGTKTVEYAPCRSQDID--------ADGQ 182

Alpha2: 196  RYCEAGFSSVVTQAGELVLGAPGGYYFLGLLAQAPVADIFSSYRPGILLWHVSSQSLSFD 255
              +C+ GFS   T+A  +-LG PG +Y+ G L    VA+I S Y P +      ++Q L+
Alpha5: 183  GFCQGGFSIDFTKADRVLLGGPGSFYWQGQLISDQVAEIVSKYDPNVYSIKYNNQ-LATR 241

Alpha2: 256  SSNPEYFDGYWGYSVAVGEFDGDLNTTEYVVGAPTWSWTLGAVEILDSY-YQRLHRLRAE 314
             ++   + D Y GYSVAVG-F+GD    ++V G P  + TLG V I D      L-    E
Alpha5: 242  TAQAIFDDSYLGYSVAVGDFNGD-GIDDFVSGVPRAARTLGMVYIYDGKNMSSLYNFTGE 300

Alpha2: 315  QMASYFGHSVAVTDVNGDGRHDLLVGAPLYMESRADRKLAEVGRVYLFLQPRGPHALGA- 373
             QMA+YFG SVA TD+NGD   D+ +GAPL+M+  +D KL EVG+V + LQ     A G
Alpha5: 301  QMAAYFGFSVAATDINGDDYADVFIGAPLFMDRGSDGKLQEVGQVSVSLQ----RASGDF 356

Alpha2: 374  PSLLLTGTQLYGRFGSAIAPLGDLDRDGYNDIAVAAPYGGPSGRGQVLVFLGQSEGLRSR 433
              +  L G +++ RFGSAIAPLGDLD+DG+NDIA+AAPYGG   +G V +F G+S GL +
Alpha5: 357  QTTKLNGFEVFARFGSAIAPLGDLDQDGFNDIAIAAPYGGEDKKGIVYIFNGRSTGLNAV 416

Alpha2: 434  PSQVLDSPFPTGS---AFGFSLRGAVDIDDNGYPDLIVGAYGANQVAVYRAQPVVKASVQ 490
             PSQ+L+  +   S   +FG-S++GA DID NGYPDLIVGA+G ++  +YRA+PV-  +
Alpha5: 417  PSQILEGQWAARSMPPSFGYSMKGATDIDKNGYPDLIVGAFGVDRAILYRARPVITVNAG 476

Alpha2: 491  LLVQDS-LNPAVKSCVLPQTKTPVSCFNIQMCVGATGHNI-PQKLSLNAELQLDRQKPRQ 548
             L V  S LN   K+C LP T   VSCFN++ C+ A G  + P+KL+   EL LD- K +
Alpha5: 477  LEVYPSILNQDNKTCSLPGTALKVSCFNVRFCLKADGKGVLPRKLNFQVELLLDKLKQKG 536

Alpha2: 549  G-RRVLLLGSQQAGTTLNLDLGGKHSPICHTTMAFLRDEADFRDKLSPIVLSLNVSLPPT 607
               RR L L S+    + N+- +       C   +A+LRDE++FRDKL+PI + +   L
Alpha5: 537  AIRRALFLYSRSPSHSKNMTISRGGLMQCEELIAYLRDESEFRDKLTPITIFMEYRLDYR 596

Alpha2: 608  EA----GMAPAVVLHGDTHVQEQTRIVLDCGEDDVCVPQLQLTASVTGSPLLVGADNVLE 663
              A    G+ P +      --  Q  I+LDCGED+VC P+L+++       + +G DN L
Alpha5: 597  TAADTTGLQPILNQFTPANISRQAHILLDCGEDNVCKPKLEVSVDSDQKKIYIGDDNPLT 656

Alpha2: 664  LQMDAANEGEGAYEAELAVHLPQGAHYMRALSNVEGFERLICNQKKENETRVVLCELGNP 723
             L + A N+GEGAYEAEL V +P  A ++  + N E   RL C  K EN+TR V+C+LGNP
Alpha5: 657  LIVKAQNQGEGAYEAELIVSIPLQADFIGVVRNNEALARLSCAFKTENQTRQVVCDLGNP 716

Alpha2: 724  MKKNAQIGIAMLVSVGNLEEAGESVSFQLQIRSKNSQNPNSKIVLLDVPVRAEAQVELRG 783
             MK   Q+   +  SV    E   SV F LQI+S N  +  S +V   V +   A VE+RG
Alpha5: 717  MKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDKVSPVVSHKVDLAVLAAVEIRG 776

Alpha2: 784  NSFPASLVVAAEEGEREQN--SLDSWGPKVEHTYELHNNGPGTVNGLHLSIHLPGQSQPS 841
              S P  + +     E +-N  + +  GP V+H YEL NNGP + +   L +  P -   +
Alpha5: 777  VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNNN 836

Alpha2: 842  DLLYILDIQPQGGLQCFPQPPVNPLKVD-WGLPIPSPSPIHPAHHKRD----RRQIFLPE 896
              LLYIL     G + C      +NPL++    L     +      +RD    +R - L E
Alpha5: 837  TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896

Alpha2: 897  PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLD--QFV 954
                     D   + C  A C  + C +  + RG+ A++ V + LW  +   +      +
Alpha5: 897  -------GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS 949

Alpha2: 955  LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALE--ERAIPIWWVLVGVLGGLLLLT 1012
             L+S A FNV   PY   P-        V T +   ++     +P+W +++ VL GLLLL
Alpha5: 950  LKSSASFNVIEFPYKNLPIEDITNSTLVTTNVTWGIQPAPMPVPVWVIILAVLAGLLLLA 1009

Alpha2: 1013 ILVLAMWKVGFFKRNRPPLEEDDEE 1037
             +LV  M+++GFFKR RPP EE + E
Alpha5: 1010 VLVFVMYRMGFFKRVRPPQEEQERE 1034
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[1] the sequence of two integrins including signal peptide 31 and 30 amino acid residues, respectively.

the cvff force field. The amino acid residues of integrin *beta*3 were fixed up. A time step of 1 fs was used during dynamics integral. The system was heated to 1000 K and retained 1 ps, and then down to 300 K to keep 50 ps. The average conformation of a series of lowest energy conformation was regarded as the preponderant conformation of the protein. Following each dynamics run, the total energy was minimized via mechanics by using a steepest descent algorithm and a subsequent conjugate gradient method. Finally, the rational model for integrin *alpha*2b*beta*3 was generated using molecular mechanics by freeing from the fixed residues (Fig 1). Comparison with human integrin αVβ3, the fit-RMS deviation of human integrin α2bβ3 is 0.6468 and 0.6424 Angstroms respectively by molecular mechanics and molecular dynamics.

Molecular modeling of the complexes with GPIIb-IIIa and its antagonist decorsin One high-resolution X-ray crystal structure of fibrinogen receptor antagonist decorsin comes from PDB database (PDB filecode 1dec)[14]. It includes 39 amino acid residues, namely APRLPQCQGD DQEKCLCNKD ECPPGQCRFP *RGD*ADPYCE. Here, the italic underlined letters *RGD* is the active sites interacting with its receptor. Based on the results that the RGD motif of fibrinogen interact with the amino-acid segment (GPIIIa: Val112-Glu171) of fibrinogen receptor, a complex model of GPIIb-IIIa with decorsin was constructed using DOCK module. Molecular dynamics and molecular mechanics were used to optimize the model as above (Fig 2).

RESULTS

Knowledge, both from the 3D structures of homologous proteins and from the general analysis of protein structure, is of value in modeling a protein of known sequence but unknown structure. Many models are constructed by homologous modeling on graphics devices, but automated procedures have come into greater use[15]. Tang *et al* used the crystal structure of bR as a template to build 3D structures of μ-opioid receptor[16]. Our group also built molecular models of human CCR5 and some interleukines with the same method[17-20]. Here we built molecular model of human fibrinogen receptor using integrin αVβ3 as the template with homologous modeling.

Structural models of human fibrinogen receptor Comparison of the structure for fibrinogen receptor with that for integrin αVβ3, they were integrin

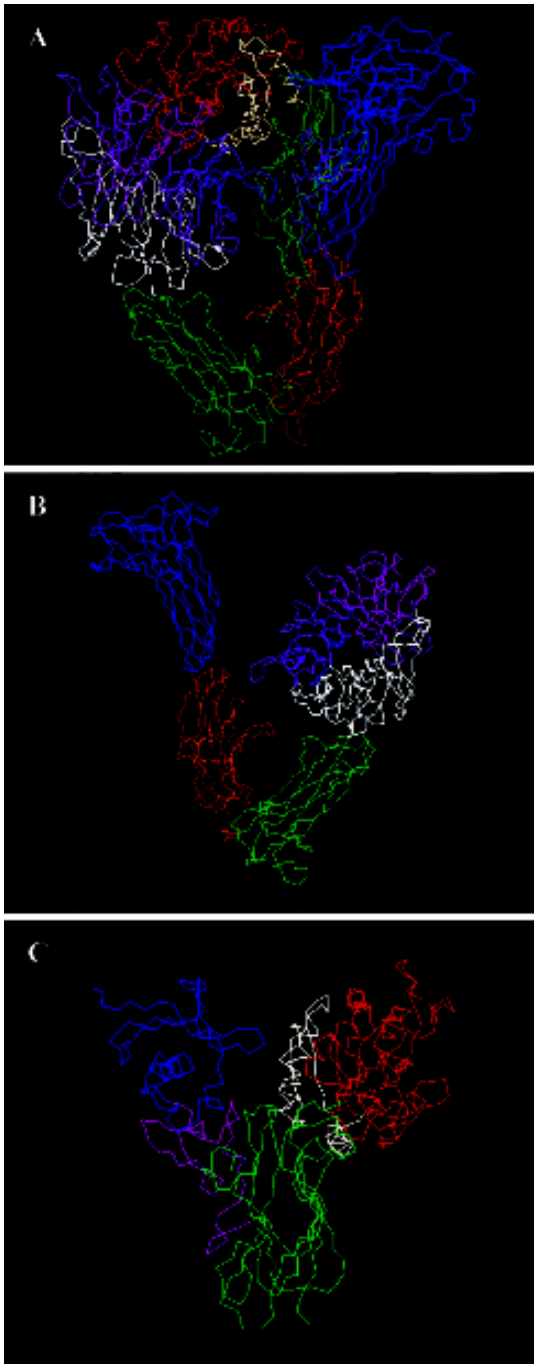

Fig 1. The structural domains of human fibrinogen receptor (GPIIb/IIIa). A) The main chain of GPIIb/IIIa appears as colored backstrone. It contains eleven structural domains, which are colored by green, cyan, red, magenta, orange, white, gray, yellow, blue, yellow-green, and purple in turn. B) The structural domains of human fibrinogen receptor GPIIb, which contains six domains. C) The structural domains of human fibrinogen receptor GPIIIa, which contains five domains. The interacting domains of GPIIa/IIIb with fibrinogen appear as red and yellow parts (in the right of C). The interacting domains of GPIIa/IIIb with decorsin appear as red parts.

heterodimeric receptors consisting of two subunits and possess a common β3 subunit, namely GPIIIa subunit. The former had 11 domains, and there were 6 domains in GPIIb (domain 1 to domain 6) and 5 domains (domain 7 to domain 11) in GPIIIa (Fig 1, Tab 2); while the latter has 12 domain[13]. There were some difference and identical points between human fibrinogen receptor and human integrin αVβ3. About the common GPIIIa subunit, domain 8 and domain 11 of fibrinogen receptorwere the same as βA-domain and βTD of integrin αVβ3. Domain 7, 9, and 10 of GPIIIa were similar to hybrid domain and EGF domain of integrin αVβ3. About GPIIb subunit of human fibrinogen receptor built based on the structure of integrin αV, domain 4, 5, and 6 of GPIIb were similar to thigh domain, calf-1 domain, and calf-2 domain of integrin αVβ3. Domain 1, 2, and 3 of GPIIb bundle up and constitute β-propeller domain of integrin αVβ3. The theoretical modeling of fibrinogen receptor was also shown to be consistent with the subunit interdomain structure. This work confirms that these integrins have interdomain structure consistent with the parallel-sandwich-hybrid topology of the subunit domain integrins.

A complex model of human fibrinogen receptor with decorsin The present survey for their interactions in the complexes with GPIIb-IIIa and its antagonist decorsin focused on the helices of GPIIb/IIIa and "U" region of decorsin. The "U" area ranging between 18 to 37 residues was inserted into the slot between helices of fibrinogen receptor (Fig 2, Tab 3). In fibrinogen receptor, the interaction sites with decorsin converged at domain 8 of the common GPIIIa. And the direct interatomic contacts were made between 16

Tab 3. The interaction between human fibrinogen receptor (GPIIb/IIIa) and its antagonist decorsin.

Residues of decorsin	Residues of fibrinogen receptor	Interaction
Asp10	GPIIIa: Asp126	Hydrogen bond
Asn18	GPIIIa: Thr182	Hydrogen bond
$Lys^{+}19$	GPIIIa: Tyr122	Hydrogen bond
$Arg^{+}28$	GPIIIa: Met124,	Hydrogen bond
	Lys125,	Hydrogen bond
	Asp251	Salt bridge
$Arg^{+}31$	GPIIIa: Met118,	Hydrogen bond
	Asp127,	Salt bridge
	Trp129,	Hydrogen bond
	Ser131,	Hydrogen bond
	Gln132,	Electrostatic
	Gln210	Electrostatic
Gly32	GPIIIa: Ser211	Hydrogen bond
Asp33	GPIIIa: Ser211	Hydrogen bond
Asp35	GPIIIa: Leu120,	Hydrogen bond
	Ser213	Hydrogen bond
Pro36	GPIIIa: Ser121	Hydrogen bond
Tyr37	GPIIIa: Tyr122,	Hydrogen bond
	Asp251	Hydrogen bond

Tab 2. The predication of structural domains of fibrinogen receptor (GPIIb/IIIa).

Domain	Comparision	Position	Sequences
1	Similar to one of β-propeller domain of GP αVβ3	GPIIb	Leu1-Trp110, Pro362-Ala450
2	Similar to one of β-propeller domain of GP αVβ3	GPIIb	Gln111-Gly242
3	Similar to one of β-propeller domain of GP αVβ3	GPIIb	Glu243-Ala361 (1 active site: Ala294-Ser316 reaction with fibrinogen gamma H12 end)
4	Similar to thigh domain of GP αVβ3	GPIIb	Gln451-Vall599
5	Similar to calf-1 domain of GP αVβ3	GPIIb	Leu600-Arg743
6	Similar to calf-2 domain of GP αVβ3	GPIIb	Ala744-Glu960
7	Similar to hybrid domain, PSI, of GP αVβ3	GPIIIa	Glu55- Pro111, Lys354-Asp434, Lys532-Asp552, Glu628-Arg636
8	Similar to βA-domain of GP αVβ3	GPIIIa	Val112-Thr286 (2 active sites: Val112-Glu171 reaction with fibrinogen alpha RGD; Val247-Gln342 reaction with fibrinogen gamma H12 end)
9	Similar to βA-domain of GP αVβ3	GPIIIa	Phe223-Cys232, Met287-Ser353 (1 active sites: Val247-Gln342 reaction with fibrinogen gamma H12 end)
10	Similar to EGF-3 and EGF-4 of GP αVβ3	GPIIIa	Trp553-Pro605
11	Similar to βTD of GP αVβ3	GPIIIa	Cys604-Thr627, Asp637-Gly690

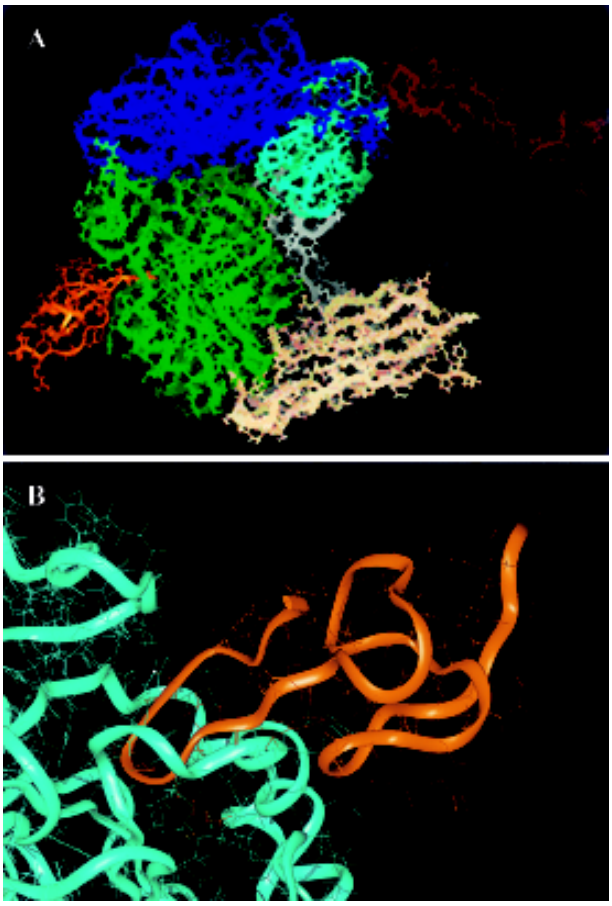

Fig 2. A complex model of GPIIa/IIIb with its antagonist decosin. A) The whole picture of GPIIa/IIIb complexed with decorsin. B) The partial interaction between GPIIa/IIIb and decorsin. The mainchain of decorsin was displayed by orange shaded ribbon. The interacting domain of GPIIa/IIIb with decorsin appear as cyan stick with shaded ribbon.

residues of fibrinogen receptor and 10 residues of decorsin by van der Waals contacts, hydrophobic interaction, electrostatic interaction, hydrogen bond, and salt bridge, respectively. Residues in contact were concentrated in four dispersed regions of human fibrinogen receptor: the RGD reaction motif — a helix composed of 118-132 residues of GPIIIa, the span from 210 to 213 of GPIIIa, Thr182 residue and Asp251 residue of GPIIIa; and they were distributed over five segments of decorsin: Asp10 residue, Asn18 and Lys19 residues, Arg28 residue, RGD motif, and Asp35-Pro36-Tyr37 segment. Nearly 50 % of the decorsin residues that make contacts human GPIIb-IIIa did so only through main-chain atoms of decorsin, and 80 % of human GPIIb-IIIa contacts were made by main-chain atoms.

Following residue ranges spans the interaction regions of decorsin with its receptor: Arg28-Asp33 (turn), Asp10-Lys19 (N-termini), and Asp35-Tyr3 7 (C-termini). Of the three regions, turn area fell most into the slot of the helices. The amidine groups of Arg28 and Arg31 residues composed a positive center. A network of hydrogen bonds maintained this twain of residues in optimal position to provide all the polar interactions to the carbohydrate: Asp251 of GPIIIa interacted with Arg28 and Asp127 interacted with Arg31 to form a salt bridge. In the contrary, the carbonyl group of Arg31 of decorsin interacted with the amide group of Gln132 and Gln210 of GPIIIa in the fashion of electrostatic interaction. Arg31 made hydrogen bonds with sulfur atom of Met118, oxygen atoms of carbonyl group of Trp129 and of Ser131 of GPIIIa, respectively. The amide group of Arg28 made hydrogen bonds with amide group of Met124 and amine group of Lys125 of GPIIIa, respectively. Gly32 and Asp33 made double hydrogen bonds with Ser211 of GPIIIa. N-terminal area protruded away from the helices of fibrinogen receptor. The amide hydrogen of Asp10 interacted with amide nitrogen of Asp126 of GPIIIa and the main chain oxygen of Asn18 interacts with the hydroxyl group of Thr182 of GPIIIa by hydrogen bond. The side chain hydrogen and main chain nitrogen of Lys19 of decorsin made two hydrogen bonds with nitrogen atom and hydroxyl group of Tyr122 of GPIIIa, respectively. At C-terminal area, there were mainly hydrogen bond interaction between decorsin and its receptor. The amide hydrogen of Asp35 interacted with amide groups of Met120 and of Ser213 of GPIIIa, respectively. Pro36 interacts with amide group of Ser121 of GPIIIa. Hydroxyl group and amide group of Tyr37 made hydrogen bonds with amide group of Asp251 and of Tyr122 of GPIIIa, respectively.

These residues in N- and C-terminal segment, together with Arg28 residue and RGD motif, form the binding pocket with its receptor. Moreover, these interactive sites of fibrinogen receptor with decorsin were similar to those of GPIIb-IIIa with fibrinogen, specially the 118 to 132 segment, which is supported by Basani RB's research results[21,22]. Maybe, decorsin has antiplatelet aggregation activity by recognizing the interactive sites of its receptor and competing with fibrinogen for interaction with its receptor GPIIb-IIIa.

DISCUSSION

Integrins are αβ heterodimer receptors that medi-

ate divalent cation-dependent cell-cell and cell-matrix adhesion through tightly regulated interactions with ligands, such as integrin αVαβ3 and α2bβ3 (GPIIb-IIIa). Moreover, integrin α2bβ3 plays the major role among platelet receptors. In resting platelets, this surface receptor is inactive and does not react with ligands, plasma proteins, fibrinogen, and von Willebrand factor, which are responsible for binding to platelets during their aggregation[23]. Besides integrin α2bβ3, there are some other specific receptors involved in functional transformation of human platelets: 1) proteinase activating receptors (PAR1 and PAR4); 2) subtype 2 purine-ergic ADP receptors (P2TAC, to inhibition of adenylate cyclase); 3) *alpha*2-adrenergic receptors (for adrenalin); 4) collagen GP VI, GP IV and integrin *alpha2beta*1 (GP Ia-IIa) receptors; 5) glycoprotein complex (GP Ib-V-IX) in which the receptor GP Ib*alpha* is specific for immobilized von Willebrand factor[24]. Receptors GPIIb-IIIa and GP Ib-V-IX not only regulate aggregation and adhesion of platelets, causing vascular occlusion; they are also involved in control of growth of thrombi and their stability. Activated platelets secrete ADP and other agonists, stimulating neighboring platelets and provoking integrin α2bβ3-mediated Ca^{2+}-dependent platelet aggregation. α2bβ3 also mediates secondary adhesion and aggregation of platelets after GP Ib-V-IX-initiated primary contact between platelets and von Willebrand factor of the vascular wall[23]. Therefore, blocking fibrinogen binding to GPIIb-IIIa can finally inhibit the final step of platelet aggregation.

Although the crystal structure of integrin αVβ3 is known, it is not the overall structure, but is only the extracellular portion of integrin αVβ3. So the model of GPIIb-IIIa built by using αVβ3 as the template is also not the whole structure. Using Domain-Ananlysis module within InsightII software, the prediction of domain of GPIIb-IIIa was made that similar to those of integrin αVβ3, the domains of each integrins assemble into an ovoid "head" and two "tails", because there is a common β3 subunit in both fibrinogen receptor and integrin αVβ3. Similar to integrin αVβ3, the main intersubunit interface lies within the head between three domains (domain 1, 2, and 3) from GPIIb and domain 8 from GPIIIa. But there are some similarities and differences between GPIIb-IIIa and integrin αVβ3.

Despite the GPIIIa subunit of GPIIb-IIIa being the same as β3 subunit of integrin αVβ3, the former contains 5 domains while the latter contains 8 domains. Domain 8 and 11 of GPIIIa are the same as βA-domain and βTD of integrin αVβ3 while domain 7, 9, and 10 of GPIIIa are similar to hybrid domain, PSI, and EGF domain of integrin αVβ3. Domain 8 consists of a central six-stranded β sheet surrounded by 8 helices. A metal ion-dependent adhesion site (MIDAS) exists in the domain 8, which is formed by the side chains of Asp119, Ser121, Ser123, Glu220, and Asp251[13] whose four residues (119, 121, 123, and 251) contribution to its interaction with decorsin. MIDAS lies adjacent to a calcium-binding site with a potential regulatory function. Adjacent to MIDAS lies a metal ion-binding sites (ADMIDAS), where there is a calcium ion because calcium is present in the crystallization buffer. Calcium is coordinated by the carbonyl oxygen of Ser123 and Met335 and by the side chains of Asp126 and Asp127[13] whose three residues (123, 126, and 127) related to decorsin.

On the other hand, GPIIb structure of GPIIb-IIIa resembles αV subunit of integrin αVβ3, which is built based on the structure of integrin αV. Similar to thigh domain, calf-1 domain, and calf-2 domain of integrin αVβ3, domain 4, 5, and 6 of GPIIb constitute a β sandwich domains. Domain 1, 2, and 3 of GPIIb are related to β-propeller domain of integrin αVβ3, and the latter consists of the former. The β-propeller is formed from the NH_2-terminal seven-fold 60 residue sequence repeats of αV and consists of seven radially arranged "blades", each formed from a four-stranded antiparallel sheet[13], which also existed in integrin αVβ3. The inner strand (strand A) of each blade lines the channel at the center of the propeller, with strands B and C of the same repeat radiating outward, and strand D of the next repeat forming the outer edge of the blade[13]. Here domain 1, domain 2, and domain 3 of GPIIb contain 3 blades, 2 blades and 2 blades, respectively. And each blade is also composed of four antiparallel strands.

Ca^{2+} is usually coordinated by oxygen atoms from side chains of residues 1, 3, 5, and 9 and the carbonyl oxygen of residue 7. The Ca^{2+}-binding sites in αVβ3 span a nine-residue segment with the consensus sequence Asp-h-Asp/Asn-x-Asp/Asn-Gly-h-x-Asp, where "h" is hydrophobic and "x" is any residue[13]. β-Propeller domain of integrin αVβ3 contains four Ca^{2+}-binding sites. And there are four Ca^{2+}-binding sites in GPIIb, whose three Ca^{2+}-binding sites are in conformity with those of αV, which are found in domain 3 (D_{297}VNGDGRHD_{305}) and domain 1 (D_{365}LDRDGYND_{373} and D_{426}IDDNGYPD_{434}), while a ten-residue segment, E_{243}FDGDLNTTE_{252}, is found in domain 3, which is

similar to another Ca^{2+}-binding site of β-propeller domain. By analogy with nine-residue segment of Ca^{2+}-binding sites, the 10-residue segment might play a role in the fashion of Ca^{2+}-binding site. The Ca^{2+}-binding loop makes extensive contacts with the domain 4. The presence of calcium is likely to make this interface more rigid.

In succession, both fibrinogen receptor and its antagonist decorsin are presented on interaction surface, which occur in domain 8 of GPIIb-IIIa. Decorsin like a shovel was inserted into an interspace between both helices, spanning from 118 to 132 of GPIIIa, namely the RGD reaction motif, and from 208 to 232 of GPIIIa, respectively. The former helix interacts with N-terminal area and turn area of decorsin while the latter helix contacts C-terminal area. Four residues (Ser121, Ser123, Asp126 and Asp127) of the RGD reaction motif contribute to MIDAS and ADMIDAS, which surround RGD motif and Asp10 of decorsin[14]. Asp251 residue of GPIIIa also contribution to MIDAS, together with the four residues above, and enclose the turn area and C-terminal area. Thus it can be seen that decorsin possesses antiplatelet aggregation activity maybe by taking up these residues, blocking calcium ion binding with ADMIDAS of GPIIIa and most inhibiting Ca^{2+}-induced platelet aggregation.

In conclusion, the present work focused on modeling of the human GPIIb-IIIa and interaction with its antagonist decorsin. Further, it is concerned with the search for all optimal positions and orientations of a set of amino acid residues of decorsin, while its binding sites include Asp10, Asn18, Lys19, Arg28, RGD motif, and Asp35-Pro36-Tyr37 segment. The related sites of human GPIIb-IIIa are mainly assembled in domain 8 (βA domain of β3 subunit) of GPIIb-IIIa, which comprises the RGD reaction motif (118-132 of GPIIIa), the span from 210 to 213 of GPIIIa, Thr182 residue and Asp251 residue of GPIIIa. Therefore, analysis of the complex between GPIIb-IIIa and decorsin provides a novel viewpoint on the structural origins of molecular recognition. And the complex models suggest that decorsin interact with its GPIIb-IIIa receptor by electrostatic, van der Waals contacts, hydrogen bond and salt bridge. This is helpful for our development and research of some new drugs, especially annexin V-guided fusion protein.

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