©2004, Acta Pharmacologica Sinica Chinese Pharmacological Society Shanghai Institute of Materia Medica Chinese Academy of Sciences http://www.ChinaPhar.com

Interaction of human fibrinogen receptor (GPIIb-IIIa) with decorsin¹

Jie YANG², Chen-yang ZHAN, Xian-chi DONG, Kun YANG, Fu-xiang WANG

State Key Laboratory of Pharmaceutical Biotechnology, Life Science College, Nanjing University, Nanjing 210093, China

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 *alphaVbeta3* (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 substratereceptor interaction model was achieved. The interaction sites with decorsin converge at domain 8 (BA domain of β 3 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 α IIb β 3), 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 (A α , B β , 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

· 1096 ·

¹ Project supported by the National Natural Science Foundation of China, No 30171094 and No 30271497.

 ² Correspondence to Dr Jie YANG. Phn 86-25-8359-4060.
 Fax 86-25-8332-4605. E-mail luckyjyj@sina.com.cn
 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 α IIb β 3 to mediate platelet aggregation^[4]. Platelet agonists (including ADP, epinephrine, thrombin, collagen, arachidonic acid, and PAF) activate aIIbb3 (inside-out) to allow the binding of soluble fibrinogen. Subsequent platelet aggregation leads to outside-in aIIb 3 signaling, which results in calcium mobilization, tyrosine phosphorylation of numerous proteins including β 3 itself, increased cytoskeletal reorganization and further activation of α IIb β 3^[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 $\beta 3$ subunit of α IIb β 3 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 NxxY₇₅₉, 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 ychain 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 alphaVbeta3 (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 alpha2b (cd41, NM-000419, NP-000410.1) and alpha5 (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 alpha2b and alpha5, similarity nearly 54 % (Tab 1). One high-resolution X-ray crystal structures of integrin *alphaVbeta3* ($\alpha V\beta 3$, PDB filecode 1JV2)^[13] was used as template structures to create integrin alpha2bbeta3 model using Homology module, where the fit-RMS deviation of subunit alpha2b with template alphaV 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 5¹⁾.

LLL P. A ND Y+CP GS FGF+DF S. R+++VGAP-T LLLSCLLPCREARNUD/DSPACSOPEGS/FGFA/DEFVPSASSMMLLVGA/KANTO 77 Alpha5: 76 PSGEETGC/FLC9WAAEGCQCSLLPD.RDETRWOSSTLQTFAAQCLCASY/SWSDYI 135 P. E. G. V. C. W+ -C. + FD L. + FK+Q. GASY S. D. I. Alpha5: 78 POIVEGGQVKLCOWSTR RCQFIEDTATOMPANDOPLE FXSIQMFCASYERSQKI 135 Alpha5: 78 Alpha5: 78 Alpha5: 78 CACAPUMWKLEXTEREAETIPVGSCF_AQPESGRAEYSFCRATLSRIVYENDESWER 195 + GACAP 1MW KETEELEATIPVGSCF_AQPESGRAEYSFCRATLSRIVYENDESWER 195 + GACAP 1MW KETEELEATIPVGSCF_LAQPESGRAEYSFCRATLSRIVYENDESWER 195 + GACAP 1MW KETEELEATIPVGSCF_AQPESGRAEYSFCRATLSRIVYENDESWER 195 + GACAP 1MW KETEELEATIPVGSCF_ACQVFFLGLLAQAPVADIFSSYRFG1LLMVSSQSLSFD 255 + GC+ GFS T+A ++ IG FC + 4 G L. VA-1 S Y P + + + Q L+ 11pha5: 136 GFCQGCFD1PTKADKVLLGGCSFYWGCDLSTUTYVYCQAPTWSWTLGAVEILDSY-YQRLHRLARE 314 + + + D Y GYSVAVG-F+GD ++ V G P + TLG V I D L- E 11pha5: 135 (MASYFG5XVATDVNCDGCRHDLLVGAPLYMESTADRKLAEVGRVVLFLQPRGPHALGA- 373 (QMAYFG5XVATDDVNCDGCRHDLLVGAPLYMESTADRKLAEVGRVVLFLQPRGPHALGA- 373 (QMAYFG5XVATDDVNCDGCRHDLLVGAPLYMESTADRKLAEVGRVVLFLQPRGPHALGA- 373 (QMAYFG5XVATDDVNCDGCRHDLLVGAPLYMESTADRKLAEVGRVVLFLQPRGPHALGA- 373 (QMAYFG5XVATDDVNCDGCRHDLLVGAPLYMESTADRKLAEVGRVVLFLQPRGPHALGA- 373 (QMAYFG5XVATDDVNCDGCRHDLLVGAPLYMESTADRKLAEVGRVVLFLQPRGPHALGA- 373 (QMAYFG5XVATDDVNCDGCRHDLDDGAPDIALAMPYGGCFKSUGL_AVKSUGL + C ++ RFGSATAPLDDDDFQHUMAAPYGCPSGRCQVLVFLQSECLESR 433 + L C +++ RFGSATAPLDDDDFQHVDLAAAPYGCGFSGRCQVLVFLQSECLESR 433 + C C +A RFGSATAPLDDDDGAPDIALAMPYGGCFKSUGLAVKSUGL Alpha5: 477 Alpha5: 577 ALTAGACGAPASELEACEAVELDJDDGAPDIALAMPYGCGFKGCAUFKLEVSUSJCAAV 416 Alpha5: 597 AADTG(QUANTCSCCCAUFAUFYVSCFUCACGAUFLANCGCUPRAINAPYCELLVKAVKSC5 59 Alpha2: 49 A NG 4: 9X E S F LQ10: ALTAGAGAUFYKSUSJLPT 4 Alpha5: 577 ALPHA2: 40 A C + P + K + N + C + V + N + N + N + N + N + N + N + N + N	Alpha2:	19	LLLGPCAAPPAWALNLDPVQLTFYAGPNGSQFGFSLDFHKDS-HGRVAIVVGAPRTLG	75
$ \begin{array}{c} \begin{array}{c} P & E \in V \in W^+ - C^+ PD \\ P \in UVEGGVLKCDWSSTR RCOPIEPDATCRN0VARDOPLE FKSUQWEGASVRSKQKI 135 \\ Alpha5: 136 \\ Alpha2: 136 \\ VACAPWGUWNVLEXTEBAEXTPVGSCF_AQPESGRAEYSPCKGNTLSRIYVENDFSWDK 195 \\ +ACAP HW & E - PVG+CFL + EV+FCR + + + + \\ Alpha5: 136 \\ FVCEAGFSSVTQAGELULAPGGYFGULAQPOSTRVEYAFCRSQDID$	Alpha5:	18		77
Alpha5: 78 PGIVEGGQVLKCDWSSTR RCQPLEFDATG/RDYAKDDPLE FKSHQWFGASVRSKQDKI 135 Alpha5: 136 VACAPWGHWNUEKTEEAEKTPVGSCFLAQPESGRARYSPCRGHLSKIYVENDFSWDK 195 +ACAP W K E = PVG-GFL + EV+FCR + + + Alpha5: 136 LACAPLYHWRTEMKQEREPVGTCFLQDGTKTVEYAFCRSQDIDADGQ 182 Alpha5: 136 LACAPLYHWRTEMKQEREPVGTCFLQDGTKTVEYAFCRSQDIDADGQ 182 Alpha5: 183 GFCQGGFS1DFTKADRVLLGAPGGYVFUGLLAQAPVADFSSVRFG1LLWHYSSGSLSFD 255 +CC +GF TA ++L D C + + C + V A + Y P + +-q 1+ Alpha5: 183 GFCQGGFS1DFTKADRVLLGGFGSFVWQQLISDQVAEIVSKYDPMYSIKYNNQ-LATR 241 Alpha5: 242 TAQAIPDDYLGTSAVACEPDGDLNTTEVVCGAPTWSWTLGAVETLDSY-YQRLHRLRAE 314 ++ D Y GYSYANG-FFGD ++V C P + TG V L L - L Alpha5: 242 TAQAIPDDYLGTSAVACEPDGDLNTTEVVCGAPTWSWTLGAVETLDSY-YQRLHRLRAE 314 Alpha5: 315 QMASYFGHSVAVTDYNGDGRHDLLVGAPLYMESRADRKLAEVGRV1FLQPRGPHALGA- 373 QMAAYFG SVA TD/HGD D ++6APL-M++ D K, EVGVY + LQ A G Alpha5: 317 PSLLLTGTQLYGRFGSAIAPLGDLDRDGYNDIAVAAPYGGFSGRGQVLFFLQPRGPHALGA- 373 QMAAYFG SVA TD/HGD D ++6APL-M++ D K, EVGVY + LQ A G Alpha5: 357 QTKLNGFVAATDINGDDAVFIGAPLEMAFSDCKLQEVGVSSLQAGGGP 356 Alpha2: 374 PSLLTGTQLYGRFGSAIAPLCDLDDGYNDIAVAAPYGGFSGRGQVLVFLGQSEGLRSR 433 + L G +++ RFGSAIAPLCDLDDGYNDIAVAAPYGGFSGRGAVLFLGQKGSUSLWF 416 Alpha5: 357 QTKLNGFVAARSWPF36VZWKGATDIDXSVFPDLIVCAGVGAVYAQAPVYKASVQ 490 PSQ+1.4 + S +FG-S+4GA DID NGYPDLIVCAGAQAVYAQAPQPVYKASVQ 490 PSQ+1.4 + S +FG-S+4GA DID NGYPDLIVCAGAQAVYAQAPQPVYKASVQ 490 PSQ+1.4 + S +FG-S+4GA DID NGYPDLIVCAGAPQAVYAQAPQPVYKASVQ 490 PSQ+1.4 + S +FG-S+4GA DID NGYPDLIVCAGAPUKALINAGKG 536 Alpha2: 491 LLVQDS-LNPAVKSCVLPQTKTPVSCFNIQUCCATGHNI-PQKLSLNAELQLDRQKPRQ 548 L V S IN K-C IP T VSCFNIQUCCATGHNI-PQKLSLNAELQLDRQKPRQ 548 L V S IN K-C IP T VSCFNIQUCCATGHNI-PQKLSLNAELQLDRQKPRQ 548 L V S IN K-C IP T VSCFNIQUCCATGHNIALSVCKRENKENVERVLEXUARGVECTAWA Alpha5: 557 TALTILLSQQACTTLNLDLGGKHSPICHTTMAFLRDEAFFRDKLPFI + L Alpha5: 557 TALTILLSQQACTTLNLDLGGKHSPICHTTMAFLRDEAFFRDKLPFI + L Alpha5: 554 ALPHA2 A G+ P + Q I +LDCCEDDVCVCQLQATASVTGSPHIPAHAVLAGVELGARPE A G+ P + Q I +LDCCEDVVCVKDLAKLSVAPGSFR	Alpha2:	76		135
+ACAP HW KE - PVG+CFL + + EY+FCR + + + + Alpha5: 136 LACAPLYHWTEMKQEREPVGTCFQ,DCTKTVEYAPCRSQID	Alpha5:	78	P E G V C W + -C + FD L+ FK+ Q GASV S D I PGIVEGGQVLKCDWSSTR RCQPIEFDATG.NRDYAKDDPLE FKSHQWFGASVRSKQDKI	135
Alpha5: 136 LACAPLYHWRTEMKQEREPVGTCFLQDGTKTVEYAPCRSQDIDADGQ 182 Alpha2: 196 RVCEAGESSVTQAGELUGAPGGYFLGLLAQAPVADIFSSYRPGILLWHVSSQDLSFD 255 +C+ GF T+A +LF G ++ +L G (++ +L G +) ++ +G) ++ 0 + Alpha5: 183 GFCQGGFSIDFTKADRVLLGGFGSFYWQGQLISDQVAEIVSKYDPNVYSIKYNNQ-LATR 241 Alpha2: 256 SSNPEYFDGYWGYSVAVGEFDGDLNTTEYVVGAPTWSWTLGAVEILDSY-YQRLHRLRAE 314 ++ + + D Y GYSVAVC-P+GD +++V G P +TIG V 1D L - E Alpha5: 242 TAQAIFDDSYLGSYAVGDFNDG-GIDDFWSGVPAARTLGAVYINDCHAUSSLYNFTCB 300 Alpha2: 315 QMASYFGHSVAVTDVNDGDGHHDLLVGAPLYMESGADRKLAEVGRVYLFLQPRGPHALGA- 373 QMAYFG SVA TD-NGD P+ CAPL+W+ +D KL EVG+V +LQ A G Alpha2: 314 QMAYFGFSVATDNDDDYAVFIGAPLFMBGSDGKLQVEQGVSVSLQRASGDF 366 Alpha2: 374 PSLLICTGLYGRFGSAIAPLCDLDRDGVNDIAVAPYGGPSGRQQVLVFLQSGCLRSR 433 + L G +++ RFGSAIAPLCDLDRDGVNDIAVAPYGGPSGRQQVLVFLQSGCLRSR 433 + L G +++ RFGSAIAPLCDLDRDGVNDIAVAPYGGPSGRQQVLVFLQSGCLRSR 434 + L G +++ RFGSAIAPLCDLDPG+NDIAIAAPYGGE 4G V +F G+S GL + Alpha5: 357 QTKLNGFEVFARFGSAIAPLCDLDPGFNDIAVAPYGGPSGRQQVLVFLQSGCLRSR 430 + L G +++ RFGSAIAPLCDLDRGGVNDIAVAPYGGPSGRQQVLVFLQSGCLRSR 430 + L G +++ +D V SURVAVIDDVGYPDLIVGAVCANQVAVRAPVVKASVQ 490 PSQ+L+ S +PG-S++GA DID NGYPDLIVGAVGANQVAVRAPVVKASVQ 490 PSQ+L+ S +PG-S++GA DID NGYPDLIVGAVGANQVAVRAPVVKASVQ 490 PSQ+L+ S +PG-S++GA DID NGYPDLIVGAVGANQVAVRAPVVKASVG 476 Alpha2: 491 LLVQDS-LPPAVKSCVLPQTKTPVSCFNIQMCVGAGGHNI-PQKLSLNAELQLDRQKPR 458 L V S LN K+C LP T VSCFN+C + A G +P4K+ E L ID - K + Alpha5: 577 AADTGLQPILLGSQQAGTLIALDLGGKMSPCHVTLKADGKGVLPRKLNFQVELLDKLQKG 538 Alpha2: 549 G-RRVLLIGSQQAGTLIALDLGGKMSPCHVTLKADGKGVLPRKLNFQVELLDKLQKG 536 Alpha2: 640 LGMDAARGEGCAVFAELAVULPGCAHYMALSNVEGFERLICNGKKENFTKVLCELOKP 723 L + A V+ C GAMPAVLIGDTHVQEQTRIVLCGCEDVCVPQLQLTASVTSSPLLAVKLDKCKG 726 Alpha2: 717 MAADTGLQPILNQFTPANISRQAHULLDGCEDVVCVPLQLQLTASVTSSPLLAVKLDKCKG 726 Alpha2: 717 MKAGTQLLACLERSVHQECKADHVERTEKDTVAGGGERNULAVKUNGLAEG 657 LVKANAQGEGAVEAELVVLPGCLQAHYMALSNVEGFERLICNGKKENFTKVVLGELGAP Alpha5: 877 TLADGTGLACHYENFEREDCQCVVQULIGSNLFATAVESVSUSSLAVLAVENER 776 Alpha2: 717 MKAGTQLLACLERSV	Alpha2:	136		195
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Alpha5:	136		182
Alpha5: 183 GPCQGGFSIDFTKADRVLLGGPCSFYWQGQLISDQVAEIVSKYDPNVYSIKYNNQ-LATR 241 Alpha2: 256 SSNPEYFDGYWCSVAVGEFDGDLNTTEYVVGAPTWSWTLGAVEILDSY-YQRLHRLRAE 314 ++++++++++++++++++++++++++++++++++++	Alpha2:	196		255
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Alpha5:	183		241
Alpha5: 242 TAQAIFDDSYLGYSVAVGDFNGD-GIDDFVSGVPRAARTLGMVYIYDGKNMSSLYNFTGE 300 Alpha5: 215 QMASYFGHSVAVTDVNGDGRHDLLVGAPLYMESRADRKLAEVGRVYLFLQPRGPHALGA- 373 QMA+TFG SVA TD+NGD D++CAPL+M++DKLEVGV++LQ A G Alpha5: 301 QMASYFGHSVATTDVNGDGRHDLLVGAPLYMESRADRKLAEVGRVYLFLQPRGPHALGA- 373 gMA+TFG SVA TD+NGD D++CAPL+M++DKLEVGV++LQ A G 41pha5: 301 QMASYFGFSVATDINGDYADVFIGAPLFMDRGSDCALQEVCQVSVLGRASGDF 356 Alpha5: 317 PSLLLTGTQLYGRFGSAIAPLGDLDPG-VDIAVAAPYGGPSGRGQVLVFLGQSEGLRSR 433 + I. G +++ RFGSAIAPLGDLD-DG-YDIA+AAPYGG +G V +F G+S GL + Alpha5: 357 QTTKLNGFEVFARFGSAIAPLGDLDDGGYDIAIAAPYGGEDKGIVYIFNGRSTGLNAV 416 Alpha2: 434 PSQVLDSPFPTGSAFGFSLRGAVDIDDNGYPDLIVGAYGANQAVAYAQPVVKASVQ 490 PSQ+L+ S +FG-S++GA DID NCYPDLIVGAFGVPAILVRARPVTTVNAG 476 Alpha2: 417 PSQILEGQWAARSMPPSFGYSMKGATDIDKNGYDLIVGAFGVDRAILVRARPVTTVNAG 476 Alpha5: 417 PSQILEGQWAARSMPPSFGYSMKGATDIDKNGYDLIVGAFGVDRAILVRARPVTTVNAG 476 Alpha5: 417 PSQILEGQWAARSMPPSFGYSMKGATDIDKNGYDLIVGAFGVDRAILVRARPVTTVNAG 476 Alpha5: 417 LEVYFSILNQDNKTCSLPCTALKVSCFNVRFCLKADGKGVLFRKLIPQVELLDKLKQKG 536 Alpha2: 549 G-RRVLLLGSQQAGTTLNLDLGGKHSPICHTTMAFLRDEADFRDKLSPIVLSLNVSLPPT 607 RR L L S+ +N+ C C +A+LRDE++FDKL+PI + L Alpha5: 557 TAADTTGLQPILNQFTRANISRGGLMQCEELIAVLRDESEFRDKLTPITIFMEYRLDYR 596 Alpha2: 608 EAGMAPAVVLHGDTHVQEQTRIVLDCGEDDVCVPQLQLTASVTGSPLLVGADNVLE 663 A G+ PQ I+LDCGEDVCVPQLQLTASVTGSPLLVGADNVLE 663 A G+ PQ I+LDCGEDVCVPQLLVASVTGSPLLVGADNVLE 663 A G+ P +Q I+LDCGEDVCVPLL+++ + + 6 DN L Alpha5: 557 LIVKAQNQGEGAYEAELVVLPQCMHWRALSNVEGFERLICNGKENETRVUCCED(PP 23 L + A NEGGAYEAELVVLPQCMHWRALSNVEGFERLICNGKKENETRVUCCED(PP 23 A h+ + SV E SV F LQI+S N + S + V + A VE+RG Alpha5: 717 VSSPDHIFLPINNEIKQEMDTSVKPLQIQISSNLFDKVSPVSHKVDLAVLAVEHG 776 Alpha2: 724 MKKNAGTGLIAGLRSVHQSGEMDTSVKPLQIQISSNLFDKVSPVSHKVDLAVLAVEHG 776 Alpha2: 714 NKKAGTGLIAGLKSVGNLEEGREGN-SDYQWITTELRNNOPSSFSKAMLILQWPYRYNNN 836 Alpha2: 897 PEQPSRLQDPVLVSCDSAPCTVVQCDLQBMARGQRAMVTVLAFLWLPSLYQRPLDQFV 954 D + C A C + NC+ + RG + H+ V + M + + F - L Alpha5: 897 TLLYILHYDIDGPMNCTSDMEINP	Alpha2:	256		314
	Alpha5:	242	TAQAIFDDSYLGYSVAVGDFNGD-GIDDFVSGVPRAARTLGMVYIYDGKNMSSLYNFTGE	300
Alpha5: 301 $QMAAYFGFSVAATDINGDDYADVFIGAPLFMDRGSDGKLQEVGQVSVSLQ=RASGDF 356$ Alpha2: 374 PSLLTGTQLYGRFGSAIAPLGDLDRGYNDIAVAAPYGGFSGGQVLVFLGQSEGLRSR 433 + L G +++ RFGSAIAPLGDLD-DG+VDIA+AAPYGG +G V +F G+S GL + Alpha5: 377 QTTKLKGFEVFARFGSAIAPLGDLDQGFNDIAIAAPYGGEDKKGIVYFLGQSEGLRSR 433 + L G +++ RFGSAIAPLGDLDQGFNDIAIAAPYGGEDKKGIVYFFKGSTGJAV 416 Alpha2: 434 PSQVLDSPFPTGSAFGFSLRGAVDIDDNGYPDLIVGAYGANQVAVYRAQPVVKASVQ 490 PSQ+L+ + S +FG-S++GA DID NGYPDLIVGAFGANQVAVYRAQPVVKASVQ 490 PSQ+L+ + S +FG-S++GA DID NGYPDLIVGAFGVDRAILYRARPVITVNAG 476 Alpha2: 417 PSQILEGQWAARSMPPSFGYSMKGATDIDKNGYPDLIVGAFGVDRAILYRARPVITVNAG 476 Alpha2: 419 LLVQDS-LNPAVKSCVLPOTKTPVSCFNIQMCVCATCHNI-PQKLSLNAELQLDRQKPRQ 548 L V S LN K+C LP T VSCFN+C + A G + P+KL+ EL LD-K + Alpha5: 477 LEVVPSILAQDNKTCJEDTAILWSCFNVRFCLKADGGGVLPRKLKNEQVELLDLKLRQG 536 Alpha2: 549 G-RRVLLGSQQAGTTINLDLGGKHSPICHTMAFLRDEADFRDKLSPIVLSLNVSLPPT 607 RR L L S + N~ + C + 4H.RDE+FRDKL+PI + L Alpha5: 537 AIRRALFLYSRSPSHSKNMISRGGLMQCEELIAYLRDESEFDKLTPITIFNCKDNVLG663 A G + P Q L+LDCGEDVCVPQLQLTASVTGSPLLVGADNUE 663 A G + P Q L+LDCGEDVCVPQLQLTASVTGSPLLVGADNUE 665 Alpha2: 664 LQMDAANEGEGAYEAELAVHLPQGAHYMRALSNVEGFERLICNQKKENETRVVLCELGNP 723 L + A N+GEGAYEAEL V +P A ++ N E RL C K EN+TR Y+C-LGNP Alpha5: 557 TAADTTGLQPILNQFTPANISRQAHILLDCGEDNVCKFKLEVSVDSDQKKIYIGDDNPLT 656 Alpha2: 664 LQMDAANEGEGAYEAELAVHLPQGAHYMRALSNVEGFERLICNQKKENETRVVLCELGNP 716 Alpha2: 724 MKKNAQIGIAMLVSVGNLEEAGESVSFQLQIRSKNSQNPNSKIVLLUPVVRAEAQVELGG 783 MK Q + + SV E SV F LQI+S N + S + V + A VE+RG Alpha5: 717 MKAGTQLLAGLRFSVHQQSEMDTSVKFDLQISSNLFDKVSPVVSHKVDLAVLAAVEIRG 776 Alpha2: 740 NSFPASLVVAAEEGEREQN-SLDSWGPVEHTYELHNNGPCTVNGLHLSIHLPGQSQPS 841 S P + + E +-N + G P V H YELNNGP + L + P + + Alpha5: 777 VSSPDHIFLPIPNWEKKEPETEEDGVPUQVHYELRNNGFSSFNALHLQWFVNNN 836 Alpha2: 842 DLLYILDIQPQGLQCFPQPPVNPLKVD-WGLPISSPIHPAHHKRDRRQIFLPE 896 LLYIL G + C + NPL++ L + HD + R - L E Alpha5: 897 TLVILHYDIGCMACTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896 Alpha2: 895 PLQSRLQDPVLVSCDSAPCTVVQCUGRLDR	Alpha2:	315		373
+ L C +++ RFCSAIAPLGDLD+DC-NDIA+APPYCC +C + F C S CL + Alpha5: 357 QTKLNGFEVFARFCSAIAPLGDLDQDCFNDIAIAAPYCC +C + F C S CL + Alpha5: 417 PSQILDSPFPTGSAFGFSLRGAVDIDDNGYPDLIVGAFGAQVAVYRAPVKASVQ 490 PSQ+L+ + S +FC-S++C A DID NCYPDLIVGAFC ++ +YRA+PV + Alpha5: 417 PSQILEGQWAARSMPPSFGYSMKGATDIDKNGYPDLIVGAFGVDRAILYRARPVITVNAG 476 Alpha2: 491 LLVQDS-LNPAVKSCVLPQTKTPVSCFNIQMCVGATCHNI-PQKLSLNAELQLDRQKPRQ 548 L V S IN K+C LP T VSCFN++ C+ A G + P+KL+ EL LD- K + Alpha5: 477 LEVYPSILNQDNKTCSLPGTALKVSCFNVRFCLKADGKGVLPRKLNFQVELLLDKLKQKG 536 Alpha2: 549 G-RRVLLCSQQAGTTLNLDLGKHSPICHTMAFLRDEADFRDKLSPIVLSLNVSLPPT 607 RR L S + N+ + C + A+LRDE+FRDKL+PI + L Alpha5: 537 AIRRALFLYSRSPSHSKNMTISRGGLMQCEELIAYLRDESEFRDKLTPITIFMEYRLDYR 596 Alpha2: 608 EACMAPAVVLHGDTHVQEQTRIVLDCGEDDVCVPQLQLTASVTGSPLLVGADNVLE 663 A G + P + Q I+LDCGEDVCVPLLVC VSDQKKIYIGDDNPLT 656 Alpha2: 604 LQMDAANEGEGAYEAELAVHLPQGAHYMRALSNVGEFRLICNQKKENETVVLCELGNP 723 L + A N=GEGAYEAEL V +P A ++ N E RL C K EN+TR V+C-LGNP Alpha5: 557 LIVKAQNQGEGAYEAELIVSIPLQADFIGVVRNNEALARLSCAFKTENQTRQVVCDLGNP 716 Alpha5: 657 LIVKAQNQGEGAYEAELIVSIPLQADFIGVVRNNEALARLSCAFKTENQTRQVVCDLGNP 716 Alpha5: 717 MKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIASSNLGPKSNIVLLDVPVRAEAQVELRG 783 MK Q + + SV E SV F LQ1+S N + S +V V + A VE+RG Alpha5: 717 MKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDKVSPVVSHKVDLAVLAAVEIRG 776 Alpha5: 717 MKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDKVSPVVSHKVDLAVLAAVEIRG 784 MK Q + + SV E SV F LQ1+S N + S +V V + A VE+RG Alpha5: 717 VSSPDHIFLPIPNWEHKENPETEEDVGPVVPLVVD-WGLPISPSPIHPAHHERDRQIFLPE 896 LLYIL G + C +NPL++ L + +RD + + L + P + + Alpha5: 837 TLLYILHYDIGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896 Alpha2: 842 DLLYILDIQPQGGLQCFPQPVPVNPLKVD-WGLPIPSPSPIHPAHHERDRQIFLPE 896 Alpha2: 897 PEQPSRLQDPVVSSLPYAVPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 1012 L'S A FKN PY P + V T + ++ C + +RD + H + L Alpha5: 950 LLSSASFNVIEFFYKNPPLEDEDEE 1037 +UV W+++CFFKR RPP EE + E	Alpha5:	301		356
Alpha5:357QTTKLNGFEVFARFGSAIAPLGDLDQDGPNDIAIAAPYGGEDKKGIVYIFNGRSTGLNAV416Alpha2:434PSQVLDSPFPTGSAFGFSLRGAVDIDDNGYPDLIVGAYGANQVAVYRAQPVVKASVQ490 $PSQ+L++S$ +FG-S++GADIDNOYPDLIVGA+G+++YRA+PV-+Alpha5:417PSQILEGQWAARSMPPSFGYSMKGATDIDKNGYPDLIVGA+G+++YRA+PV-+Alpha5:417PSQILEGQWAARSMPPSFGYSMKGATDIDKNGYPDLIVGA+G+++YRA+PV-+Alpha5:417PSQILEGQWAARSMPPSFGYSMKGATDIDKNGYPDLIVGA+G +++YRA+PV-+Alpha2:491LLVQDS-LNPAVKSCVLPQTKTPVSCFNIQCCGATGHNI-PQKLSLNAELQLDRQKPRQ548L.VS.NK+C LP TVSCR++A GP+KL+EL LDK K+Alpha5:417LEVYPSILNQDNKTCSLPGTALKVSCFNVRFCLKADGKGVLPRKLNFQVELLLDKLKQKG536Alpha2:608EAGMAPAVVLHCDTHVQEQTRIVLDCGEDDVCVPQLQLTASVTGSPLLVGADNVLE608EAGMAPAVVLHCDTHVQEQTRIVLDCGEDDVCVPQLQLTASVTGSPLLVGADNVLE619ha2:608EAGMAPAVVLHCDTHVQEQTRIVLCGEDDVCVPQLQLTASVTGSPLLVGADNVLE610Alpha5:5977AADTTGLQPILNQFTPANISRQAHILLDCGEDNVCKPKLEVSVDSDQKKIYIGDDNPLT656657LIVKAQNQGEGAYEAELAVHLPQCAHYMRALSNVEGFERLICNQKKENETRVVLCELGNP718Alpha5:717MKKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDKVSPVVSHKVDLAVLAVELAVEIRG776Alpha5:774Alpha5:777VSSPDHIFLPIPNWEHKENPETEEDDGPVVPHIVELNNGPSSPSKAMLLQWPVKTNNN836Alpha2:841S P + +S P + +E +N + +877VSSPDHIFLPIPNWEHKENPETEEDDGPVVPHIVELNNGPSSPSKAMLHLQWPYKTNNN836 <td< td=""><td>Alpha2:</td><td>374</td><td></td><td>433</td></td<>	Alpha2:	374		433
$\begin{array}{llllllllllllllllllllllllllllllllllll$	Alpha5:	357		416
Alpha5:417PSQILEGQWAARSMPPSFGYSMKGATDIDKNGYPDLIVGAFGVDRAILYRARPVITVNAG476Alpha2:491LLVQDS-LNPAVKSCVLPQTKTPVSCFNIQMCVGATGHNI-PQKLSLNAELQLDRQKPRQ548LVS LNK+C LPVSCFN++ C+A6+ P+KL+EL D-K+Alpha5:477LEVYPSILNQDNKTCSLPGTALKVSCFNVRFCLKADGKGVLPRKLNFQVELLLDKLKQKG536Alpha2:549G-RRVLLLCSQQAGTTLNLDLGGKHSPICHTTMAFLRDEADFRDKLSPIUSLINSLEPPT607RRLL<++N++C	Alpha2:	434		490
L V S LN K+C LP T VSCFN++ C+ A G + P+KL+ EL LD-K + Alpha5: 477 LEVYPSILNQDNKTCSLPGTALKVSCFNVRFCLKADGKGVLPRKLNFQVELLLDKLKQKG 536 Alpha2: 549 G-RRVLLLGSQQAGTILNLDLGGKHSPICHTTMAFLRDEADFRDKLSPIVLSLNVSLPPT 607 RR L L S+ +N+ + C +A+LRDE++FRDKL+PI + + L Alpha5: 537 AIRRALFLYSRSPSHSKMTISRGGLMQCEELIAYLRDESEFRDKLTPITIFMEYRLDYR 596 Alpha2: 608 EAGMAPAVVLHGDTHVQEQTRIVLDCGEDDVCVPQLQLTASVTGSPLLVGADNVLE 663 A C+ P + - Q I+LOGEDVC P+L++ + + G N L Alpha5: 597 TAADTTGLQPILNQFTPANISRQAHILLDCGEDDVCKPKLEVSVDSDQKKIYIGDDNPLT 656 Alpha2: 664 LQMDAANEGECAYEAELAVHLPQCAHYMRALSNVEGFERLICNQKKENETRVVLCELCNP 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 + A VE+RG Alpha5: 717 MKAGTQLLAGLFRSVHQQSEMDTSVKFDLQIQSSNLFDKVSPVVSHKVDLAVLAAVEIRG 776 Alpha2: 784 NSFPASLVVAAEEGEREQN-SLDSWGPKVEHTYELHNNGPGTVNGLHLSIHLPGQSQS 841 S P + + E +N + GP V+H YEL NNGP + L + P + + Alpha5: 777 VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNNN 836 Alpha2: 842 DLLYILDIQPQGGLQCFPQPPVNPLKVD-WGLPIPSPSPIHPAHEKRDRRQIFLPE 896 LLYIL G + C +NPL++ L + RD +R - L E Alpha5: 837 TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGGGERDHLITKRDLALSE 896 Alpha2: 897 PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV 954 D + C A C + C + RG+ A++ V + UW + + + Alpha5: 897GDIHTLGCVAQLKLKVQVGRLDRGKSAILVYKSLLWTEFFNNKENQNHSYS 949 Alpha2: 955 LQSHAWFNVSSLPYAVPLSLPRGEAQVWTQLLRALEERAIPIWWLVGVLGGLLLT 1012 L+S A FNV P P + V T + + + + + CLLL Alpha5: 950 LKSSASFNVIEFPKKN.PPLEEDDTINSTLVTTNTWGTIQPAPMPVPVWWIILAVLAGLLLLA 1009 Alpha2: 1013 ILVLAMWKVGFFKRNRPPLEEDDEE 1037 +UV M+++GFFKR RPP EE + E	Alpha5:	417		476
Alpha5:477LEVYPSILNQDNKTCSLPGTALKVSCFNVRFCLKADGKGVLPRKLNFQVELLLDKLKQKG536Alpha2:549G-RRVLLLGSQQAGTTLNLDLGGKHSPICHTTMAFLRDEADFRDKLSPIVLSLNVSLPPT607 RR L L S+ + N+ + C + A+LRDE++FRDKL+PI + + LAlpha5:537AIRRALFLYSRSPSHSKNMTISRGGLMQCEELIAYLRDESEFRDKLTPITIFMEYRLDYR596Alpha2:608EAGMAPAVVLHGDTHVQEQTRIVLDCGEDDVCVPQLQLTASVTGSPLLVGADNVLE663 A G+ P + Q I+LDCGEDHVC P+L++ + +G D LAlpha5:597TAADTTGLQPILNQFTPANISRQAHILLDCGEDNVCKPKLEVSVDSDQKKIYIGDDNPLT656Alpha2:664LQMDANEGEGAYEAELAVHLPQAHYMRALSNVEGFERLICNQKKENETRVVLCELGNP723 L + A N+GEGAYEAEL V +P A ++ N E RL C K EN+TR V+C-LGNPAlpha5:657LIVKAQNQGEGAYEAELIVSIPLQADFIGVVRNEALARLSCAFKTENQTRQVVCDLGNP716Alpha5:717MKKNAQIGIAMLVSVGNLEEAGESVSFQLQIRSKNSQNPNSKIVLLDVPVRAEAQVELRG783 MK Q+ + SV E SV F LQI+S N + S +V V + A VE+RGAlpha5:717MKGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDKVSPVVSHKVDLAVLAAVEIRG776Alpha2:784NSFPASLVVAAEEGEREQNSLDSWGPKVEHTYELHNNCPGTVNGLHLSIHLPGQSQPS841 S P ++ E ++N + GP V+H YEL NNGP ++ L + P + +Alpha5:777VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNN836Alpha2:842DLLYILDIQPQGGLQCFPQPVNPLKVD-WGLPIPSPSPIHPAHHKRDRRQIFLPE896 LLYIL G + C + MPL++ L + + RD +R + L EAlpha5:837TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE896Alpha5:837TLLYILHYDIDGPMNCTSDAEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE896Alpha2:841 D + C A C + C + + RG+A++ V LW + + +4Alpha	Alpha2:	491		548
RR L L S+ $+ + + + + + + + + + + + + + + + + + + $	Alpha5:	477		536
A1pha5:537AIRRALFLYSRSPSHSKNMTISRGGLMQCEELIAYLRDESEFRDKLTPITIFMEYRLDYR596A1pha2:608EAGMAPAVVLHGDTHVQEQTRIVLDCGEDDVCVPQLQLTASVTGSPLLVGADNVLE663 A G+P+ Q I+LDCGED+VC P+L+++ + +G DN LA1pha5:597TAADTTGLQPILNQFTPANISRQAHILLDCGEDNVCKPKLEVSVDSDQKKIYIGDDNPLT656A1pha2:664LQMDAANEGEGAYEAELAVHLPQGAHYMRALSNVEGFERLICNQKKENETRVVLCELCNP723 L + A N+GEGAYEAEL V +P A ++ N E RL C K EN+TR V+C-LCNPA1pha5:657LIVKAQNQGEGAYEAELIVSIPLQADFIGVVRNNEALARLSCAFKTENQTRQVVCDLGNP716A1pha5:657LIVKAQNQGEGAYEAELIVSIPLQADFIGVVRNNEALARLSCAFKTENQTRQVVCDLGNP716A1pha5:717MKKNAQIGIAMLVSVGNLEEAGESVSFQLQIRSKNSQNPNSKIVLLDVPVRAEAQVELRG783 MK Q+ + SV E SV F LQI+S N + S +V V + A VE+RGA1pha5:717MKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDKVSPVVSHKVDLAVLAAVEIRG776A1pha2:784NSFPASLVVAAEEGEREQNSLDSWGCPKVEHTYELHNNGPGTVNGLHLSIHLPGQSQPS841 S P ++ E +-N ++ GP V+H YEL NNGP ++ L + P -+ +A1pha5:777VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNNN836A1pha2:842DLLYILDIQPQGGLQCFPQPPVNPLKVD-WGLPIPSPSPIHPAHHKRDRRQIFLPE896A1pha2:897PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV954 D + C A C + C + + RG+ A++ V + LW + + +A1pha5:897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS949A1pha2:955LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT1012 L+S A FNV PY P- V T + ++ +P+W +++ VL GLLLLA1pha2:950LKSSASFNVIEFPYKNLPIEDITNSTLVTTNVTWGIQPAPMPVPVWVIILAVLAG	Alpha2:	549		607
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 NSFPASLVVAAEEGEREQNSLDSWGPKVEHTYELHNNGPGTVNGLHLSIHLPGQSQPS 841 S P ++ E ++N ++ GP V+H YEL NNGP ++ L + P + + Alpha5: 777 VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNNN 836 Alpha2: 842 DLLYILDIQPQGGLQCFPQPPVNPLKVD-WGLPIPSSPSIPHPAHHKRDRRQIFLPE 896 LLYIL G + C +NPL++ L + + RD +R + L E Alpha5: 837 TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896 Alpha2: 897 PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV 954 D + C A C + C + + RG + A++ V + LW + + + + Alpha5: 897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILVVKSLLWTETFMNKENQNHSYS 949 Alpha2: 955 LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 1012 L+S A FNV PY P+ V T + ++ +PW +++ VL GLLLL Alpha5: 950 LKSSASFNVIEFPYKNLPIEDITNSTLVTTNVTWGIQPAPMPVPVWVIILAVLAGLLLLA 1009 Alpha2: 1013 ILVLAMWKVGFFKRNPPLEEDDEE 1037 +LV M+++GFFKR RPP EE + E	Alpha5:	537		596
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 MKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDRVSPVVSHKVDLAVLAAVEIRG 776 Alpha2: 784 NSFPASLVVAAEEGEREQN-SLDSWGPKVEHTYELHNNGPGTVNGLHLSIHLPGQSQPS 841 S P ++ E \pm -N ++ GP V+H YEL NNGP ++ L + P + + Alpha5: 777 VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNNN 836 Alpha2: 842 DLLYILDIQPQGGLQCFPQPPVNPLKVD-WGLPIPSPSPIHPAHHKRDRRQIFLPE 896 LLYIL G + C +NPL++ L + + RD +R + L E Alpha5: 837 TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896 Alpha2: 897 PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV 954 D + C A C + C + + RG + A++ V + LW + + + Alpha5: 897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS 949 Alpha2: 955 LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 1012 L+S A FNV PY P+ V T + ++ +PW +++ VL GLLLL Alpha5: 950 LKSSASFNVIEFPYKNLPIEDITNSTLVTTNVTWGIQPAPMPVPVWVIILAVLAGLLLLA 1009 Alpha2: 1013 ILVLAMWKVGFFKRNPPLEEDDEE 1037 +LV M+++GFFKR RPP EE + E	Alpha2:	608		663
L + A N+GEGAYEAEL V +P A ++ + N E RL C K EN+TR V+C-LGNP A1pha5: 657 LIVKAQNQGEGAYEAELIVSIPLQADFIGVVRNNEALARLSCAFKTENQTRQVVCDLGNP 716 A1pha2: 724 MKKNAQIGIAMLVSVGNLEEAGESVSFQLQIRSKNSQNPNSKIVLLDVPVRAEAQVELRG 783 MK Q+ + SV E SV F LQI+S N + S +V V + A VE+RG A1pha5: 717 MKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDKVSPVVSHKVDLAVLAAVEIRG 776 A1pha2: 784 NSFPASLVVAAEEGEREQNSLDSWGPKVEHTYELHNNGPGTVNGLHLSIHLPGQSQPS 841 S P + + E ++N + + GP V+H YEL NNGP + + L + P + + A1pha5: 777 VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNNN 836 A1pha2: 842 DLLYILDIQPQGGLQCFPQPPVNPLKVD-WGLPIPSPSPIHPAHHKRDRRQIFLPE 896 LLYIL G + C +NPL++ L + +RD +R + L E A1pha5: 837 TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896 A1pha2: 897 PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV 954 D + C A C + C + + RG+ A++ V + LW + + + A1pha5: 897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS 949 A1pha2: 955 LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 1012 L+S A FNV PY P+ V T + ++ +P+W +++ VL GLLLL A1pha5: 950 LKSSASFNVIEFPYKNLPIEDITNSTLVTTNVTWGIQPAPMPVPVWVIILAVLAGLLLLA 1009 A1pha2: 1013 ILVLAMWKVGFFKRNRPPLEEDDEE 1037 +LV M+++GFFKR RPP EE + E	Alpha5:	597		656
 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 NSFPASLVVAAEEGEREQNSLDSWGPKVEHTYELHNNGPGTVNGLHLSIHLPGQSQPS 841 S P + + E ++N + + GP V+H YEL NNGP + + L + P + + Alpha5: 777 VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNNN 836 Alpha2: 842 DLLYILDIQPQGGLQCFPQPPVNPLKVD-WGLPIPSPSPIHPAHHKRDRRQIFLPE 896 LLYIL G + C +NPL++ L + +RD +R + L E Alpha5: 837 TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896 Alpha2: 897 PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV 954 D + C A C + C + + RG + A++ V + LW + + + Alpha5: 897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS 949 Alpha2: 955 LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 1012 L+S A FNV PY P+ V T + ++ +P+W +++ VL GLLLL Alpha2: 1013 ILVLAMWKVGFFKRNRPPLEEDDEE 1037 +LV M+++GFFKR RPP EE + E 	Alpha2:	664		723
MK Q+ + SV E SV FLQI+S N + S +V V + A VE+RG Alpha5: 717 MKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDKVSPVVSHKVDLAVLAAVEIRG 776 Alpha2: 784 NSFPASLVVAAEEGEREQNSLDSWGPKVEHTYELHNNGPGTVNGLHLSIHLPGQSQPS 841 S P + + E + N + GP V + H YEL NNGP + + L + P + + Alpha5: 777 VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNNN 836 Alpha2: 842 DLLYILDIQPQGGLQCFPQPPVNPLKVD-WGLPIPSPSPIHPAHHKRDRRQIFLPE 896 LLYIL G + C + NPL++ L + + RD + R + L E Alpha5: 837 TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896 Alpha2: 897 PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV 954 D + C A C + C + RG+ A++ V + LW + + + Alpha5: 897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS 949 Alpha2: 955 LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 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:	657		716
 Alpha5: 717 MKAGTQLLAGLRFSVHQQSEMDTSVKFDLQIQSSNLFDKVSPVVSHKVDLAVLAAVEIRG 776 Alpha2: 784 NSFPASLVVAAEEGEREQNSLDSWGPKVEHTYELHNNGPGTVNGLHLSIHLPGQSQPS 841 S P + + E + + P + + GP V+H YEL NNGP + + L + P + + Alpha5: 777 VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNNN 836 Alpha2: 842 DLLYILDIQPQGGLQCFPQPPVNPLKVD-WGLPIPSPSPIHPAHHKRDRRQIFLPE 896 LLYIL G + C + NPL++ L + + RD + R + L E Alpha5: 837 TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896 Alpha2: 897 PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV 954 D + C A C + C + + RG + A++ V + LW + + + Alpha5: 897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS 949 Alpha2: 955 LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 1012 L+S A FNV PY P+ V T + + + P+W +++ VL GLLLL Alpha2: 1013 ILVLAMWKVGFFKRNRPPLEEDDEE 1037 +LV M+++GFFKR RPP EE + E 	Alpha2:	724		783
$\begin{array}{llllllllllllllllllllllllllllllllllll$	Alpha5:	717		776
 Alpha5: 777 VSSPDHIFLPIPNWEHKENPETEEDVGPVVQHIYELRNNGPSSFSKAMLHLQWPYKYNNN 836 Alpha2: 842 DLLYILDIQPQGGLQCFPQPPVNPLKVD-WGLPIPSPSPIHPAHHKRDRRQIFLPE 896 LLYIL G + C +NPL++ L + +RD +R + L E Alpha5: 837 TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896 Alpha2: 897 PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV 954 D + C A C + C + + RG + A++ V + LW + + + Alpha5: 897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS 949 Alpha2: 955 LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 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 	Alpha2:	784		841
LLYIL G + C +NPL++ L + +RD +R + L E A1pha5: 837 TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE 896 A1pha2: 897 PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV 954 D + C A C + C + + RG+ A++ V + LW + + + + A1pha5: 897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS 949 A1pha2: 955 LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 1012 L+S A FNV PY P+ V T + ++ +P+W +++ VL GLLLL A1pha5: 950 LKSSASFNVIEFPYKNLPIEDITNSTLVTTNVTWGIQPAPMPVPVWVIILAVLAGLLLLA 1009 A1pha2: 1013 ILVLAMWKVGFFKRNRPPLEEDDEE 1037 +LV M+++GFFKR RPP EE + E	Alpha5:	777		836
Alpha5:837TLLYILHYDIDGPMNCTSDMEINPLRIKISSLQTTEKNDTVAGQGERDHLITKRDLALSE896Alpha2:897PEQPSRLQDPVLVSCDSAPCTVVQCDLQEMARGQRAMVTVLAFLWLPSLYQRPLDQFV954 D + C A C + C + + RG+ A++ V + LW + + + + Alpha5:897Alpha5:897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS919Alpha2:955LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT1012 L+S A FNV PY P+ V T + ++ +P+W +++ VL GLLLLAlpha5:950LKSSASFNVIEFPYKNLPIEDITNSTLVTTNVTWGIQPAPMPVPVWVIILAVLAGLLLLA1009Alpha2:1013ILVLAMWKVGFFKRNRPPLEEDDEE1037 +LV M+++GFFKR RPP EE + E	Alpha2:	842		896
D + C A C + C + + RG+ A++ V + LW + + + Alpha5: 897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS 949 Alpha2: 955 LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 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:	837		896
Alpha5: 897GDIHTLGCGVAQCLKIVCQVGRLDRGKSAILYVKSLLWTETFMNKENQNHSYS 949 Alpha2: 955 LQSHAWFNVSSLPYAVPPLSLPRGEAQVWTQLLRALEERAIPIWWVLVGVLGGLLLLT 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	Alpha2:	897		954
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:	897		949
Alpha5: 950 LKSSASFNVIEFPYKNLPIEDITNSTLVTTNVTWGIQPAPMPVPVWVIILAVLAGLLLLA 1009 Alpha2: 1013 ILVLAMWKVGFFKRNRPPLEEDDEE 1037 +LV M+++GFFKR RPP EE + E	Alpha2:	955		1012
+LV M+++GFFKR RPP EE + E	Alpha5:	950		1009
	Alpha2:	1013		
	Alpha5:	1010		

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 beta3 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 alpha2bbeta3 was generated using molecular mechanics by freeing from the fixed residues (Fig 1). Comparison with human integrin $\alpha V\beta 3$, the fit-RMS deviation of human integrin $\alpha 2b\beta 3$ 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 highresolution 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 ECPPG-QCRFP <u>RGD</u>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 $\alpha V\beta 3$ as the template with homologous modeling.

Structural models of human fibrinogen receptor Comparison of the structure for fibrinogen receptor with that for integrin $\alpha V\beta 3$, 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 β 3 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 $\alpha V\beta 3$. About the common GPIIIa subunit, domain 8 and domain 11 of fibrinogen receptorwere the same as β A-domain and β TD of integrin $\alpha V\beta 3$. Domain 7, 9, and 10 of GPIIIa were similar to hybrid domain and EGF domain of integrin $\alpha V\beta 3$. 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 $\alpha V\beta 3$. Domain 1, 2, and 3 of GPIIb bundle up and constitute β -propeller domain of integrin $\alpha V\beta 3$. 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	Residues of fibrinogen	Interaction	
decorsin	receptor		
A 10		TT 1 1 1	
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).

Domai	n Comparision	Position	Sequences
1	Similar to one of β -propeller domain of GP $\alpha V\beta 3$	GPIIb	Leu1-Trp110, Pro362-Ala450
2	Similar to one of β -propeller domain of GP $\alpha V\beta 3$	GPIIb	Gln111-Gly242
3	Similar to one of β -propeller domain of GP $\alpha V\beta 3$	GPIIb	Glu243-Ala361 (1 active site: Ala294-Ser316 reaction with fibrinogen gamma H12 end)
4	Similar to thigh domain of GP $\alpha V\beta 3$	GPIIb	Gln451-Vall599
5	Similar to calf-1 domain of GP $\alpha V\beta 3$	GPIIb	Leu600-Arg743
6	Similar to calf-2 domain of GP $\alpha V\beta 3$	GPIIb	Ala744-Glu960
7	Similar to hybrid domain, PSI, of GP $\alpha V\beta 3$	GPIIIa	Glu55- Pro111, Lys354-Asp434, Lys532-Asp552, Glu628-Arg636
8	Similar to βA -domain of GP $\alpha V\beta 3$	GPIIIa	Val112-Thr286 (2 active sites: Val112-Glu171 reaction with fibrinogen alpha RGD; Val247-Gln342 reaction with fibrinogen gamma H12 end)
9	Similar to β A-domain of GP α V β 3	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 $\alpha V\beta 3$	GPIIIa	Trp553-Pro605
11	Similar to βTD of GP $\alpha V\beta 3$	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 re-

gions of decorsin with its receptor: Arg28-Asp33 (turn), Asp10-Lys19 (N-termini), and Asp35-Tyr3 7 (Ctermini). 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 Cterminal 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 $\alpha\beta$ heterodimer receptors that medi-

ate divalent cation-dependent cell-cell and cell-matrix adhesion through tightly regulated interactions with ligands, such as integrin $\alpha V \alpha \beta 3$ and $\alpha 2b\beta 3$ (GPIIb-IIIa). Moreover, integrin $\alpha 2b\beta 3$ 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 α 2b β 3, 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) alpha2-adrenergic receptors (for adrenalin); 4) collagen GP VI, GP IV and integrin alpha2beta1 (GP Ia-IIa) receptors; 5) glycoprotein complex (GP Ib-V-IX) in which the receptor GP Ibalpha 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 $\alpha 2b\beta 3$ -mediated Ca²⁺dependent platelet aggregation. $\alpha 2b\beta 3$ 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 $\alpha V\beta 3$ is known, it is not the overall structure, but is only the extracellular portion of integrin $\alpha V\beta 3$. So the model of GPIIb-IIIa built by using $\alpha V\beta 3$ 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 $\alpha V\beta 3$, the domains of each integrins assemble into an ovoid "head" and two "tails", because there is a common $\beta 3$ subunit in both fibrinogen receptor and integrin $\alpha V\beta 3$. Similar to integrin $\alpha V\beta 3$, 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 $\alpha V\beta 3$.

Despite the GPIIIa subunit of GPIIb-IIIa being the same as β 3 subunit of integrin $\alpha V\beta$ 3, the former contains 5 domains while the latter contains 8 domains. Domain 8 and 11 of GPIIIa are the same as β A-domain and β TD of integrin α V β 3 while domain 7, 9, and 10 of GPIIIa are similar to hybrid domain, PSI, and EGF domain of integrin $\alpha V\beta 3$. 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 $\alpha V\beta 3$, which is built based on the structure of integrin αV . Similar to thigh domain, calf-1 domain, and calf-2 domain of integrin $\alpha V\beta 3$, 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 $\alpha V\beta 3$, and the latter consists of the former. The β -propeller is formed from the NH₂-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 $\alpha V\beta 3$. 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.

Ca²⁺ is usually coordinated by oxygen atoms from side chains of residues 1, 3, 5, and 9 and the carbonyl oxygen of residue 7. The Ca²⁺-binding sites in $\alpha V\beta 3$ 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 $\alpha V\beta 3$ contains four Ca²⁺-binding sites. And there are four Ca²⁺-binding sites in GPIIb, whose three Ca²⁺-binding sites are in conformity with those of αV , which are found in domain 3 (D₂₉₇VNGD-GRHD₃₀₅) and domain 1 (D₃₆₅LDRDGYND₃₇₃ and D₄₂₆IDDNGYPD₄₃₄), while a ten-residue segment, E₂₄₃FDGDLNTTE₂₅₂, is found in domain 3, which is similar to another Ca²⁺-binding site of β -propeller domain. By analogy with nine-residue segment of Ca²⁺-binding sites, the 10-residue segment might play a role in the fashion of Ca²⁺-binding site. The Ca²⁺-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 Ca²⁺-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 Vguided fusion protein.

REFERENCES

1 Senti M, Aubo C, Bosch M, Pavesi M, Pena A, Masia R, et

al. Platelet glycoprotein IIb/IIIa genetic polymorphism is associated with plasma fibrinogen levels in myocardial infarction patients. Clin Biochem 1998; 31: 647-51.

- 2 Schulman SP, Goldschmidt-Clermont P J, Topol E J, Califf RM, Navetta FI, Willerson JT, *et al.* Effects of integrelin, a platelet glycoprotein IIb/IIIa receptor antagonist, in unstable angina. A randomized multicenter trial. Circulation 1996; 94: 2083-9.
- 3 Menegatti M, Asselta R, Duga S, Malcovati M, Bucciarelli P, Mannucci PM, *et al.* Identification of four novel polymorphisms in the Alpha and gamma fibrinogen genes and analysis of association with plasma levels of the protein. Thromb Res 2001; 103: 299-307.
- 4 Law DA, Nannizzi-Alaimo L, Phillips DR. Outside-in integrin signal transduction. αIIbβ3- (GPIIb/IIIa) tyrosine phosphorylation induced by platelet aggregation. J Biol Chem 1996; 271: 10811-5.
- 5 Wu H, Peng L, Li J, Wu W, Hou Q, Liu H, *et al.* Reversible exposure of human platelet fibrinogen receptors by antiplatelet tetraspanin monoclonal antibodies via induction of a conformational change in membrane glycoprotein IIb/IIIa complex. Thromb Res 1999; 95: 245-53.
- 6 Jenkins AL, Nannizzi-Alaimo L, Silver D, Sellers JR, Ginsberg MH, Law DA, *et al*. Tyrosine phosphorylation of the β3 cytoplasmic domain mediates integrin- cytoskeletal interactions. J Biol Chem 1998; 273: 13878-85.
- 7 Law DA, DeGuzman FR, Heiser P, Ministri-Madrid K, Killeen N, Phillips DR. Integrin cytoplasmic tyrosine motif is required for outside-in αIIbβ3 signaling and platelet function. Nature 1999; 401: 808-11.
- 8 Parise LV. Integrin αIIbβ3 signaling in platelet adhesion and aggregation. Curr Opin Cell Biol 1999; 11: 597-601.
- 9 Weller T, Alig L, Muller MH, Kouns WC, Steiner B. Fibrinogen receptor antagonists- a novel class of promising antithrombotics. Drugs Future 1994; 19: 461-76.
- 10 Seymour JL, Henzel WJ, Nevins B, Stults JT, Lazarus RA. Decorsin, a potent glycoprotein IIb/IIIa antagonist and platelet aggregation inhibitor from the leech Macrobdella decora. J Biol Chem 1990; 265: 10143-7.
- 11 Topol EJ, Byzove TV, Plow EF. Platelet GPIIb/IIIa blockers. Lancet 1999; 353: 227-31.
- 12 Polverino de Laureto P, Scaramella E, De Filippis V, Marin O, Doni MG, Fontana A. Chemical synthesis and structural characterization of the RGD-protein decorsin: a potent inhibitor of platelet aggregation. Protein Sci 1998; 7: 433-44.
- 13 Xiong JP, Stehle T, Diefenbach B, Zhang R, Dunker R, Scott DL, *et al.* Crystal Structure of the extracellular segment of integrin alpha V/beta 3. Science 2001; 294: 339-46.
- 14 Krezel AM, Wagner G, Seymour-Ulmer J, Lazarus RA. Structure of the RGD protein decorsin: conserved motif and distinct function in leech proteins that affect blood clotting. Science 1994; 264: 1944-7.
- 15 Johnson MS, Srinivasan N, Sowdhamini R, Blundell TL. Knowledge-Based Protein Modeling. Biochem Mol Biol 1994; 29: 1-68.
- 16 Tang Y, Chen KX, Jiang HL, Ji RX. Molecular modeling of mu opioid receptor and its interaction with ohmefentanyl.

 \cdot 1104 \cdot

Acta Pharmacol Sin 1996; 17: 156-60.

- 17 Yang J, Liu CQ. Molecular modeling on human CCR5 receptors and complex with CD4 antigens and HIV-1 envelope glycoprotein gp120. Acta Pharmacol Sin 2000; 21: 29-34.
- 18 Yang J, Liu CQ. Interaction between human interleukin-16 and CD4 receptor of HIV-1. Acta Pharmacol Sin 2000; 21: 547-53.
- 19 Yang J, Zhang YW, Huang JF, Zhang YP, Liu CQ. Structure analysis of CCR5 from human and primates. J Mol Struct Theochem 2000; 505: 199-210.
- 20 Yang J, Liu CQ. Molecular modeling on some human interleukins sharing gamma c of interleukin-2 receptor, and structure-function relationships. J Mol Struct Theochem 2000; 532: 1-10.
- 21 Basani RB, D'Andrea G, Mitra N, Vilaire G, Richberg M,

Kowalska MA, *et al.* RGD-containing peptides inhibit fibrinogen binding to platelet alpha(IIb)beta3 by inducing an allosteric change in the amino-terminal portion of alpha(IIb). J Biol Chem 2001; 276: 13975-81.

- 22 Basani RB, French DL, Vilaire G, Brown DL, Chen F, Coller BS, *et al.* A naturally occurring mutation near the amino terminus of alpha IIb defines a new region involved in ligand binding to alpha IIb beta 3. Blood 2000; 95: 180-8.
- 23 Stattil SJ. Signaling through platelet integrin alpha IIb beta 3: inside-out, outside-in, and sideways. Thromb Haemost 1999; 82: 318-25.
- 24 Basanoval AV, Baskoval IP, Zavalova LL. Vascular-Platelet and Plasma Hemostasis Regulators from Bloodsucking Animals. Biochemistry (Mosc) 2002; 67: 143-50.