Integrin β-subunits contain an N-terminal PSI (for plexin-sema- phorin-integrin) domain that contributes to integrin activation and harbors the PI(Ⅲ) alloantigen associated with immune thrombocytopenias and susceptibility to sudden cardiac death. Here we report the crystal structure of PSI in the context of the crystallized α3β1 ectodomain. The integrin PSI forms a two-stranded antiparallel β-sheet flanked by two short helices; its long interstrand loop houses PI(Ⅲ) and may face the EGF2 domain. The integrin PSI contains four cysteine pairs connected in a 1-4, 2-8, 3-6, 5-7 pattern. An unexpected feature of the structure is that the final, eighth cysteine is located C-terminal to the Ig-like hybrid domain and is thus separated by the hybrid domain from the other seven cysteines of PSI. This architecture may be relevant to the evolution of integrins and should help refine the current models of integrin activation.

Integrins are heterodimeric (αβ) cell-matrix and cell-cell adhesion receptors, with each subunit containing a large extracellular domain, a single-pass transmembrane domain, and a short cytoplasmic tail (1). Integrins are often expressed on the cell surface in an inactive state (unable to bind physiologic ligands) but can be rapidly activated by intracellular signals (inside-out activation) (2). Once liganded, integrins cluster and initiate outside-in signals similar to classical receptors that modify cellular functions. The precise mechanism of integrin activation is incompletely understood.

Insights into structure-activity relationships in integrins were greatly aided by our determination of the crystal structure of the ectodomain from integrin α3β1 alone and in complex with the prototypical ligand RGD (3, 4). The structure has four domains in the α3 subunit: an N-terminal seven-bladed propeller followed by an Ig-like “thigh” domain and two co-linear β-sandwich domains calf-1 and calf-2. The β-subunit ectodomain consists of eight domains. The N-terminal PSI domain (5) is followed by an Ig-like “hybrid” domain (with the ligand-binding vWFA domain (6)A emerging from the loop connecting its two β-sheets). The hybrid domain is then connected to four EGF1-like domains and a novel β-tail domain (60). An unexpected feature of the crystal structure is that α3β1 is gnel- flexed at the α3 and β2 “knees” such that the head abuts the legs. Current models suggest that a straightening of the genu is required for physiologic ligand binding (in the switch-blade model of activation (6)) or for ligand-induced outside-in signaling (in the deadbolt model (6)). A better understanding of the basis of integrin activation requires structure determination of the activation-sensitive domains PSI, EGFL1, and EGFL2 (reviewed in Ref. 7), which are all located in the genuflexed β-subunit but were not resolved in the published structure. Although some features of the PSI domain, including two short helices, were visible in our electron density maps, our main chain tracing was inconsistent with published cysteine pairing (8) and predictions (9) especially between Cys6 and Cys435. Since several cysteines cluster close together, we were initially unable to build the domain with certainty. The density for EGFL1 and EGFL2 was even less well defined.

The ~50-amino acid PSI domain, first recognized based on primary sequence alignments, is present in one or more copies in more than 500 proteins (see smart.embl-heidelberg.de/smart/get_members.pl?WHAT=NDB_COUNT&NAME=PSI). It is most commonly found in plexins, semaphorins, and integrins, glycoproteins that mediate cell growth, migration, and differentiation. Two recently determined structures of semaphorin 4D (SEMA4D) (9) and the plexin MET (10), each containing a PSI domain, have now allowed us to build the integrin PSI domain into our α3β1 maps without ambiguity. The salient and unexpected features of this structure as it relates to integrin architecture, activation, and disease are presented and discussed here.
A Novel Adaptation of the Integrin PSI Domain

**Architectural and Disease Implications**—The integrin PSI domain contributes to integrin activation as evidenced by binding of activation-sensitive monoclonal antibodies such as AP5 (which binds the N-terminal six amino acids of PSI of $\beta_3$ (14)) and activating amino acid substitutions (15, 16) such as an alanine substitution of the Cys435 of $\beta_3$, located at the C-terminus of the hybrid domain, is an integral part of the integrin PSI (Fig. 2A). Thus the hybrid domain is an insertion into the last loop of PSI. This unexpected architecture through which the PSI and hybrid domains are connected may explain the incorrect predictions of cysteine pairing of the PSI domain in integrins (5, 8). The hybrid A/B loop contains an arginine (Arg93), conserved between integrin $\beta$-chains, that contributes to the interface with the PSI domain.

**Functional and Disease Implications**—The integrin PSI domain contributes to integrin activation as evidenced by binding of activation-sensitive monoclonal antibodies such as AP5 (which binds the N-terminal six amino acids of PSI of $\beta_3$ (14)) and activating amino acid substitutions (15, 16) such as an alanine substitution of the Cys435 of $\beta_3$, located at the C-terminus of the hybrid domain, is an integral part of the integrin PSI (Fig. 2A). Thus the hybrid domain is an insertion into the last loop of PSI. This unexpected architecture through which the PSI and hybrid domains are connected may explain the incorrect predictions of cysteine pairing of the PSI domain in integrins (5, 8). The hybrid A/B loop contains an arginine (Arg93), conserved between integrin $\beta$-chains, that contributes to the interface with the PSI domain.

**Structural features of the integrin PSI domain.** A, stereo view of the averaged experimental density maps obtained through MAD and SIRAS phasing (3), with the PSI domain chain trace. The map is contoured at 1.1 $\sigma$. The domain is represented with a C-alpha tracing, with the disulfide bonds shown. Cys$^{13}$ is labeled and connected by density to Cys$^{435}$. B, ribbon drawing showing the structure of the integrin PSI domain from $\beta_3$, with a two-stranded $\beta$-sheet (labeled A and B, red) flanked by two helices (blue). The side chain of the core Trp$^{25}$ (yellow) and cysteine pairing are shown. Selected amino acids (G1, P2, N3, T4, L33, A50, and C435) are labeled (see "Results and Discussion" for details). Residues 51–53 (not included in the trace) are indicated by a green dotted line. The black dotted line points to the position of EGF1. The lower portion of the hybrid domain (gray) and its Cys$^{435}$-Cys$^{433}$ bond, which immediately precedes the Cys$^{33}$-Cys$^{35}$ bond, are shown. In this and the following figure, $n$ and $c$ denote respectively the N and C termini of PSI. C, ribbon drawing of the $\alpha_v\beta_\alpha$ ectodomain, including PSI. The $\alpha_v$ chain is shown in blue and the $\beta_\alpha$ in red. The $\alpha_v\beta_\alpha$ domains (excluding EGF1 and EGF2) are labeled.

Recent structure determinations of two PSI domains (9, 10), allowed us to establish the $\beta_3$ PSI fold without ambiguity (Fig. 1A). We fitted the cysteine cores of SEMA4D and MET structures into our maps, and this allowed us to establish points of reference for the PSI domain fold. It became clear that Cys$^{13}$, and not the published Cys$^{33}$, pairs with Cys$^{435}$ (Fig. 1B). It also became clear that the region around Cys$^{435}$ substitutes for a portion of the PSI domain seen in the SEMA4D and MET structures, and thus Cys$^{435}$ is integral to PSI. We were able to build residues 1–50 of the PSI domain by using the corrected disulfide bond pattern. The linker between PSI and hybrid domains (residues 51–53) is not well defined in our density maps and was not included. In our efforts to connect the PSI domain with the hybrid domain, we also noticed that the N-terminal strand of the hybrid domain was out of register by one residue. We have corrected this error, which has no bearing on previously published interpretations of $\alpha_v\beta_\alpha$ structure and function. The location of the PSI domain in relation to the rest of the $\alpha_v\beta_\alpha$ ectodomain is shown in Fig. 1C.
of microvascular injury (19). It has also been reported that the PlA2 allele is a risk factor for acute renal allograft rejection (20). The PSI domain also contributes to the binding of certain drugs that trigger drug-dependent antibodies to αmβ3, precipitating thrombocytopenia (21).

The present crystallographic studies clarify a number of observations about structure-activity relationships in integrins. First, they establish the correct pairing of cysteines and domain boundaries for the integrin PSI. This will allow a reinterpretation of many functional studies and will be invaluable in devising new ones to evaluate the salient features of the structure. Second, the structure reveals that the N terminus of the activation-sensitive AP5 epitope is solvent-exposed (Fig. 1 B), which may explain the ability of the AP5 monoclonal antibody to bind the ectodomain in solution (not shown). The Cys98-Cys105 disulfide bridge contributes to the PSI/hybrid interface and likely helps to restrict movement of the PSI with respect to the hybrid domain. The activating effect of the Cys105 to alanine substitution is expected to make this interface more flexible, and it may also allosterically alter putative contacts between PSI and EGF1/2, thus accounting for the activating nature of this mutation. Interruption of the adjacent Cys406. Cys433 disulfide bond in the hybrid domain may have a similar functional outcome. Third, Leu33 is located in a hydrophobic segment of the distinctively long AB loop (between strands A and B of the PSI domain, Fig. 1B). Its replacement with Pro, a conformationally restricted residue, may alter the structure of this loop leading to an autoantibody response and immune thrombocytopenia. Elucidation of the three-dimensional structure of the PlA1 alloantigen may be useful in averting the autoimmune consumption of platelets through drug design.

The present structure reveals that all three residues are located on the same side of the integrin. Finally, it is now apparent that two insertions have contributed to the current architecture of the integrin β-subunit, one in the last loop of PSI and the second in between the two sheets of the hybrid domain, setting the stage both for formation of the integrin heterodimer and its regulated ability to bind physiologic ligands.

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FIG. 2. Structure and sequence alignments of the PSI domains. A, best superposition of the PSI domains from β3 (red), SEMA4D (light blue), and MET (dark blue). The cysteine pairs are shown in yellow. Note that Cys355 of β3 is equivalent to the last cysteine in the other two structures. B, sequence alignment and cysteine pairing of the PSI domain from all eight human integrin β-subunits and that from SEMA4D, MET, KIAA0315 (a semaphorin), EGFL4 (multiple EGF-like-domain protein 4), attractin, and VSPR (receptor for viral-encoded semaphorin protein), the latter four were included to optimize the sequence alignment. Positions of the hybrid and EGF1 domains are shown. Cysteines are in red. Note that Cys8 and Cys2 are not found in semaphorins. Residue numbers in the primary sequence are shown. The secondary structure elements (cylinders for helices and arrows for strands) of the hybrid and EGF1 domains are shown. The italicized eighth cysteine (Cys355 in β3), considered part of PSI in integrins, is separated by a single residue (italicized) from EGF1.
A Novel Adaptation of the Integrin PSI Domain Revealed from Its Crystal Structure
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