Integrins are modular (αβ) heterodimeric proteins that mediate cell adhesion and convey signals across the plasma membrane. Interdomain motions play a key role in signal transduction by propagating structural changes through the molecule, thus controlling the activation state and adhesive properties of the integrin. We expressed a soluble fragment of the human integrin β2 subunit comprising the plexin-semaphorin-integrin domain (PSI)/hybrid domain/I-EGF1 fragment and present its crystal structure at 1.8-Å resolution. The structure reveals an elongated molecule with a rigid architecture stabilized by nine disulfide bridges. The PSI domain is located centrally and participates in the formation of extended interfaces with the hybrid domain and I-EGF1 domains, respectively. The hybrid domain/PSI interface involves the burial of an Arg residue, and contacts between PSI and I-EGF1 are mainly mediated by well conserved Arg and Trp residues. Conservation of key interacting residues across the various integrin β subunits sequences suggests that our structure represents a good model for the entire integrin family. Superposition with the integrin β3 receptor in its bent conformation suggests that an articulation point is present at the linkage between its I-EGF1 and I-EGF2 modules and underlines the importance of this region for the control of integrin-mediated cell adhesion.
fragment of the integrin β2 subunit consisting of the PSI, hybrid, and I-EGF1 domains and present a high resolution structure of these three domains (hereafter abbreviated PHE1). The PSI domain is located centrally in the structure and participates in the formation of extended interfaces with both the hybrid domain and I-EGF1 domains, respectively. The structure brings an important missing piece to the picture of the extracellular portion of the integrin receptor by suggesting that these three domains act as a rigid unit and that an articulation point is located between the I-EGF1 and I-EGF2 modules of the integrin.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—The cDNA coding for PHE1 was constructed from the wild type human β2 cDNA by removing the region encoding residues Lys339–Asn359 corresponding to the O-like domain insertion, and by adding a hexahistidine tag to facilitate purification followed by a stop codon after Glu660, which marks the C-terminal end of the I-EGF1 domain (17, 18) (Fig. 1A). The PCR product was inserted into the pcDNA3 expression vector, and the plasmid was transfected into 293 cells. Stable cell lines were created by selection for G418. A single clone with high PHE1 expression was grown in Dulbecco’s modified Eagle’s culture medium (JRH Biosciences). The integrin β2 subunit consisting of the PSI, hybrid, and I-EGF1 domains and present a high resolution structure of these three domains (hereafter abbreviated PHE1).

Crystallization and Data Collection—

Crystallographic studies were carried out at the European Synchrotron Radiation Facility (Grenoble, France). A potassium tetrachloroplatinate (K2PtCl4) derivative was collected on an R-EXpress Image Plate detector using CuKα radiation from a MicroMax-007 rotating anode operating at 20 mA and 40 kV. Data were processed with the programs MOSFLM and SCALA (19). Crystal parameters and data collection statistics are summarized in Table I.

Structure Determination and Refinement—The structure was determined by the single isomorphous replacement method using anomalous scattering (SIRAS) from a K2PtCl4 derivative. Two heavy atom binding sites were located using the program SOLVE (20), and an initial map was calculated after solvent flattening. This initial map allowed the tracing of most of the main chain atoms of the hybrid and PSI domains. Phases calculated from the partial model combined with the experimental SIRAS phases were used to calculate a new map that was modified using the program DM (19). The whole procedure was iterated, and the envelope was modified. Once about 70% of the residues had been traced, the program ARP/WARP (19) was used for final phase improvement and model building. Phasing and refinement statistics are presented in Table II. The model was refined by slow cool energy minimization and B-factor refinement protocols of the program CNS (22) with the Engh and Huber force field constants (23) using the maximum likelihood amplitude target. Manual inspection and correction of the model were made using the program O (24). The free R-factor was calculated from

| Table I | Data collection and phasing statistics |
|---------|--------------------------------------|
| **Native** | K2PtCl4 |
| Soaking concentration/time | 1 mM; 12 h |
| Wavelength (Å) | 0.97600 |
| Cell parameters (Å), P2₁ | a = 58.60, b = 31.82, c = 74.95 |
| a = 58.60, b = 31.40, c = 75.54 |
| β = 91.20° | β = 91.90° |
| Resolution range (Å) | 30–1.80 | 19.6–2.80 |
| No. of observed reflections | 182,914 | 26,551 |
| No. of unique reflections⁻¹ | 25,996 (3,671) | 7,034 (702) |
| Completeness (%) | 99.5 (97.0) | 99.8 (98.4) |
| Multiplicity | 7.0 (6.1) | 3.8 (3.8) |
| Rmerge | 0.068 (0.478) | 0.088 (0.289) |
| I/σ(I) | 19.2 (3.2) | 7.8 (3.0) |
| Solvent content (%) | 55.2 | 55.0 |
| No. of sites | 2, 0.70, 0.75 |
| Rmerge (centrics, acentrics) | 1.33, 1.47 |
| Phasing power | 0.38 |

⁻¹ The numbers in parentheses refer to the last (highest) resolution shell.

| Table II | Refinement statistics |
|---------|----------------------|
| **Native** | |
| Resolution range (Å) | 20.0–1.80 |
| Intensity cutoff (F(000)) | 0 |
| No. of reflections, completeness (%) | 24,602, 98.1 |
| Used for refinement | 23,569 |
| Used for Ryp calculation | 1,233 |
| No. of non hydrogen atoms | |
| Protein | 1,657 |
| Missing residues | 4 |
| Sugar residues | 2 |
| Water molecules | 219 |
| B-factor (°) | |
| Rfree (integrated) (%) | 23.37 |
| Rfree (calc) (%) | 25.29 |
| r.m.s. deviations from ideality | |
| Bond lengths (Å) | 0.0049 |
| Bond angles (°) | 1.30 |
| Ramachandran plot | |
| Residues in most favored regions (%) | 87.9 |
| Residues in additional allowed regions (%) | 11.5 |
| Overall G factor | 0.22 |

⁻¹ Rmerge = Σ||Fcalc|| − |Fobs||/Σ|Fcalc|, where Fcalc is the calculated heavy atom structure factor for acentric and centric reflections, respectively.

⁻² Mean value of figure of merit before density modification and phase combination.

RESULTS

Protein Expression—The integrin β2 subunit can be expressed in the absence of any integrin α subunits on COS-7 cells but can only be detected by monoclonal antibodies whose epitopes map to regions outside the I-like domain (15). This result suggests that the proper folding of the I-like (βΔ) domain

5% of the measured unique data, randomly chosen, that were not included in the refinement. The coordinates and structure factors have been deposited in the Protein Data Bank with code 1YUK. Surface area calculations were carried out with the program AREAIMOL (19) with a radius of the probe sphere of 1.7 Å. Figs. 2 and 4–6 were drawn using the program Pymol (written by Dr. Delano, available at pymol.sourceforge.net).

PSI/Hybrid Domain/I-EGF1 β₂ Integrin Structure

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should be noted that the epitopes of KIM89, MEM148, KIM202, 7E4, and H52 have been mapped to the domains in the PHE1 fragment of the specific monoclonal antibodies: lane 1, MEM148; lane 2, KIM99; lane 3, MMH24; lane 4, MEM48; lane 5, 7E4; lane 6, KIM202; lane 7, H52. It should be noted that the epitopes of KIM89, MEM148, KIM202, 7E4, and H52 have been mapped to the domains in the PHE1 fragment of the β2 integrin subunit (S. K. A. Lescar, unpublished results; and Ref. 15), that of MEM48 has been mapped to I-EGF2 and I-EGF3 (18), and that of MHM24 has been mapped to the integrin αε subunit (15). C, SDS-PAGE analysis of PHE1 on a 15% gel before (B) and after (A) treatment with peptide N-glycosidase F. Molecular masses of standard protein markers are shown (M).

Overall Architecture—The PHE1 segment adopts an elongated rodlike structure with overall dimensions of 82 × 30 × 30 Å. The structure is organized around three β-strands, βX, βA', and βG, which form a central platform with the middle strand βA' connecting back to strand βB in the hybrid domain, while strands βX and βG insert into the split PSI domain (see Figs. 1 and 2). The PSI domain is located centrally in the structure and makes extensive interactions with both the hybrid and I-EGF1 domains. Thus, the structure appears to be rather rigid and is further stabilized by nine disulfide bonds. A superposition of the PSI/hybrid tandem with the equivalent domains in the αεβ1 integrin structure (Protein Data Bank code 1TYE) gives a r.m.s. deviation of 2.2 Å for 169 equivalent Cα atoms. This illustrates that the relative orientation is well preserved between the hybrid and PSI domains of βA and βG integrins, an observation in agreement with the good conservation of the residues that form the interface (see below).

The Hybrid Domain—The N- and C-terminal sequences flanking the I-like (βA) domain of integrin β2 (Fig. 1A) cluster in space and form the hybrid domain that adopts a β-sandwich fold. In the PHE1 construct, the I-like (βA) subunit is absent, and the 4 residues connecting to it are not resolved in the electron density map and were not included in the current model. A total of 219 well defined water molecules as well as two N-linked GlcNAc residues (Fig. 2A) and 2 histidine residues from the C-terminal His6 tag were placed (Fig. 1A). All residues from the model fall into favorable or allowed regions of the Ramachandran diagram (Table I).

Quality of the Model—The final model refined at 1.80-Å resolution includes 58 residues of the PSI domain, 120 residues of the hybrid domain, and 34 residues of the I-EGF1 domain (Fig. 2). The 4 residues (Ala-Lys-Leu-Ser) that connect the hybrid domain to the N- and C-terminal portions of the I-like (βA) domain insertion could not be traced in the electron density maps. Mass spectrometry and SDS-PAGE analysis of dissolved protein crystals rules out proteolytic cleavage at this position. Thus, these residues are presumably mobile. Likewise 5 residues in the glycine-rich loop connecting strands βX with βA of the hybrid domain are not visible in the electron density map and were not included in the current model. A total of 219 well defined water molecules as well as two N-linked GlcNAc residues (Fig. 2A) and 2 histidine residues from the C-terminal His6 tag were placed (Fig. 1A). All residues from the model fall into favorable or allowed regions of the Ramachandran diagram (Table I).
superimposes well with the corresponding domain of the β3 integrin with a r.m.s deviation of 1.6 Å for 115 equivalent Cα atoms. This suggests that the I-like (βA) subunit does not influence the conformation of the hybrid domain and that other protein modules could be inserted at the same position, opening the possibility of creating chimeric receptor molecules. The loop connecting the βX and βA strands is significantly shorter in integrin β2 compared with the corresponding loops in β1, β2, β5, β6, and β7 integrins (Figs. 2 and 3) and is presumably flexible. In the β2 integrin structure, the same loop is longer and is stabilized through contacts with residues from the I-like (βA) domain (3, 9).

The PSI Domain—The PSI domains of integrin β2 and β3 have similar structures (4, 9) with a residual r.m.s. deviation between the two domains of 1.8 Å for 54 equivalent Cα atoms after superposition. All four disulfide bridges, including the one between the second cysteine residue (Cys11 in integrin β2) and the cysteine following the hybrid domain (Cys425), are conserved and superimposable. However, differences exist between the two molecules. The N-terminal helix α1 present in β3 is missing in integrin β2, and an additional 3/10 helix and an α-helix, α3a, are present (Fig. 2A). An N-linked carbohydrate structure is present at Asn28 currently modeled as a single GlcNAc residue, although there is indication of an additional branched sugar residue.

The I-EGF1 Domain—The I-EGF1 domain of the integrin β subunits was not resolved in previous structural studies, and our data provide the first view at atomic resolution of this domain. Unlike the integrin-EGF domains 2, 3, and 4 as well as those of the TIED (ten β-integrin EGF-like repeat domains) protein (17, 25) that have 8 cysteine residues, the I-EGF1 structure β2 subunit has only a total of 6 cysteines engaged in three disulfide bonds (Figs. 3 and 4). The structure of I-EGF3 domain of the β2 subunit was determined by NMR, and the 8 cysteines were shown to arrange in the C1-C5, C2-C4, C3-C6, C7-C8 pattern (11). Amino acid sequence alignment shows that I-EGF1 belongs to this group of EGF domains but with the C2-C4 disulfide pair missing and not to the laminin EGF domains that have the C1-C3, C2-C4, C5-C6, C7-C8 disulfide arrangement (17). A comparison with the I-EGF3 structure of the β2 integrin (11) and the I-EGF4 structure of the β3 integrin (3) reveals that the absence of the second and fourth cysteine is accompanied by a large movement of the polypeptide chain (including the basic patch formed by Arg428, Arg432, and Arg434) that projects away from the central β-sheet formed by strands β1 and β2 (Fig. 4A). This is presumably partly due to the release of the structural constraint brought by the C2-C4 disulfide bridge in the I-EGF3 domains, which is absent in I-EGF1. Interestingly the closest structural homologue of I-EGF1 is the EGF domain of P-selectin (26) whose polypeptide chain follows a similar path with 33 equivalent residues superimposed with an r.m.s. deviation of 1.7 Å (Fig. 4A).

Interaction between Domains—The interactions between the PSI domain with the hybrid and I-EGF1 domains are stabilized through the formation of extensive interfaces of 815 and 1099 Å², respectively. The interface between the PSI and hybrid domains in the integrin subunits are similar with a comparable buried surface area of 860 Å² and the involvement of a highly invariant Arg residue in multiple contacts with main chain atoms of the PSI domain in both cases (Fig. 5). The side chain of Arg66 (Arg93 in integrin β3 (9)) is deeply buried in the PSI/hybrid interface forming four hydrogen bonds with main chain atoms from Gly18, Pro19, Cys21, and Pro29. A water molecule trapped in the interface mediates an additional interaction between the carbonyl oxygen of the conserved Pro67 with the PSI domain. Thus the hybrid and PSI domains are held in a rigid orientation with respect to each other in both the β2 and β3 integrins, and given the strict conservation of the Arg residues, the relative orientation of these two domains is likely to be conserved in all integrins (Figs. 3 and 4). The PSI domain and I-EGF1 domains are also found to interact extensively. A list of interactions is given in Table III. Hydrogen bonds are formed between the guanido group of Arg30 and the carbonyl oxygen of Gly546 and between Trp25 of the PSI domain and the carbonyl group of Cys445. Interestingly in addition to Cys445, the other 3 residues, namely Trp25, Arg30, and Gly446, are

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**Fig. 2.** A, overall view of the PHE1 molecule with its secondary structure elements labeled. In the “upright” conformation of the integrin, the ligand binding domain would be located at the top, and the plasma membrane would be located at the bottom of the figure. The nine disulfide bridges and the two N-linked acetylglucosamine residues resolved in the structure are represented as sticks and labeled. The positions of the I-like domain (or βA) insertion in the native integrin structure between β-strands βB and βC of the hybrid domain is indicated. B, representative final electron density map calculated with phases from the refined model displayed at a contour level of 1σ showing the N-linked N-acetylglycosamine residue at Asn94.
Fig. 3. Alignment of sequences (obtained from GenBank™) of human integrin β subunits. Secondary structure elements are marked above the alignment in green for the PSI domain, yellow for the hybrid domain, and red for the I-EGF1 domain of the β₂ subunit. The structural elements for the integrin β₂ PSI and hybrid domains are marked in blue. Cysteines are highlighted in yellow. The conserved residues contributing
highly invariant among the β integrin sequences (Fig. 3), suggesting that the interface between PSI and I-EGF1 may also be well conserved. The compactness of the structure is further strengthened by the formation of the Cys1-Cys6 and Cys4-Cys10 disulfide bonds that maintain the potentially mobile linker region 423–427 of the PSI domain in close proximity with the bulk of the PSI and I-EGF1 domains, respectively (Fig. 2).

DISCUSSION

Taken together, the structural data presented here suggest that the PSI, hybrid, and I-EGF1 domains represent a rather rigid unit that may act as a lever to orientate the I-like (βA) domain with respect to the lower leg. However, the current evidence is partly based on the close match between the hybrid/PSI domains in only two experimentally determined structures (4, 9), and more data are needed to resolve this issue. In contrast to the β3 integrin, the α-helix α1 is missing in our PHE1 β2 structure. Rather than at its N terminus, the polypeptide chain of the β2 integrin PSI domain adopts an extended conformation. It would be interesting to see whether this conformation is preserved in the presence of an α subunit partner and the flanking I-EGF domains. Indeed a number of structural modifications are expected in the PSI and I-EGF1 domains in the context of an α subunit and the flanking I-EGF2 domain due to inter- and intrachain interactions. In particular, the assessment of the existence or not to the contacts between the PSI and hybrid domains are highlighted in light blue, and those between the PSI and I-EGF1 domains are in pink. The nine disulfide pairs are marked with arrows. The two N-glycosylation sites are indicated by red inverted triangles. Amino acid sequence GenBank accession numbers, labeled as ITB (integrin β), are as follows: β1, 124965; β2, 124966; β3, 124968; β4, 13638154; β5, 124970; β6, 13432176; β7, 124973; β8, 4504779.
of a fourth β4 strand in the I-EGF1 module, which would form hydrogen bonds with the currently unpaired β3 strand (see Figs. 2 and 4), must await the structural determination of an integrin fragment comprising both the I-EGF1 and I-EGF2 domains. Although the disulfide linkages are conserved in the integrin structures determined so far (3, 9, 11), several pairs of cysteine residues are found in close vicinity (e.g. Cys11-Cys425 is spatially close to Cys427-Cys445, and Cys3-Cys21 is close to Cys4-1-Cys445, see Fig. 2). Given small adjustment in the three-dimensional structure, their sulfhydryl groups are at the right distance to form alternate disulfide bonds. Thus, we cannot completely rule out the possibility of an alternative disulfide arrangement that would introduce subtle alterations in the structure, possibly acting as relays to transduce signals. In our structure, which is devoid of the domains C-terminal to the I-EGF1, Ile455 is largely exposed to the solvent at the lower end of the molecule (Fig. 5). A hydrophobic residue is also found at the same position in integrins β1, β3, β5, and β2 (Fig. 3). It is thus probable that Ile455 of I-EGF1 makes contact with some hydrophobic residues protruding from the flanking I-EGF2 domain. Having defined the relative orientation of the hybrid, PSI, and I-EGF1 domains, what can we infer about the probable location of the I-EGF2 fragment? We performed a superposition of the PSI/hybrid domains of the PHE1 structure with the corresponding domains resolved in the bent conformation of the integrin αβ2 structure (4) (Fig. 6). Based on the resulting locations of the C-terminal end of the I-EGF1 domain and the N-terminal end of the I-EGF3, we observed that to join the I-EGF1 and I-EGF2 segments together, these two modules must associate in antiparallel fashion, presumably exposing the long hydrophilic stretch present between cysteines C1 (Cys461) and C2 (Cys470) of the I-EGF2 module of the β2 integrin subunit at the tip of the molecule in its bent conformation (Fig. 6). This observation is in agreement with the extended model proposed for the I-EGF2/I-EGF3 tandem based on NMR data (9). Two monoclonal antibodies that stimulate ligand binding to integrin αβ2 recognize epitopes located at the N terminus of the PSI domain, further emphasizing the importance of this region of the integrin molecule for signal transduction (16). In addition, a pathogenic hantavirus recognizes the PSI domain in its bent conformer, and this interaction was suggested to disturb vascular permeability by restricting integrin dynamics (27). Recent reports suggest that the bent conformers of α5β3 (28) and α6β2 (29) can interact with ligands under appropriate conditions. These data point to the existence of several integrin conformers displaying subtle variations in terms of their activation states, thus emphasizing the importance of studies aimed at defining these structural states and how they correlate with integrin activation.

In conclusion, one critical region for the transmission of structural changes with functional significance is located at the junction of the PSI, hybrid, and I-EGF1 domains with a linkage between I-EGF1 and I-EGF2 possibly acting as a conformational switch between the head and the legs of the β subunit of the integrin receptor. Our result point to this linker as an important region for regulating integrin-mediated cell adhesion. This hypothesis can now be tested by raising antibodies directed against peptides connecting the I-EGF1 and I-EGF2 modules and assessing their ability to interfere with integrin activation.

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